Bio-inspired Magnetite Mineralization in Gelatin Hydrogels: A Small Angle Scattering Investigation

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Preamble

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Zusammenfassung


Stichwort: Biomineralisation, Magnetit, Gelatine, Hydrogelen, SANS, VSANS, SAXS
Abstract

Biomineralization represents a sophisticated process of forming a highly hierarchically ordered mineral structure by a living organism. The process is carried out under strict biological control of specially designed biomacromolecules. Mineralization mechanisms permitting such sophistication control typically involve interaction between an inorganic mineral and an organic matrix interface. A clear understanding of the mechanisms of this process may pave way for exploration of new material design strategies and generation of materials with improved mechanical, chemical and physical properties. We thus attempt to follow nature’s fabrication strategy of using biomolecules and study the mineralization process in-situ which might shed light on the mechanistic aspects of controlling the process.

The aim of this work is to investigate, understand and probably control the mechanism of bio-inspired magnetite mineralization in organic matrices and the organic-inorganic hybrid structures by Small Angle Neutron and X-ray Scattering methods. The studies focus on the nucleation and growth of the magnetite particles in the gelatin hydrogel matrix by employing biomineralization strategies from three natural bio-minerals. The SANS contrast matching method was used by the variation of heavy water content in the H$_2$O/D$_2$O mixture in order to emphasize the structure of the individual components of the complex material on the different stages of biomineralization process. These results have provided structural information and understanding of the mechanisms of magnetite mineralization as well as in-situ. Several hypotheses have been introduced to explain functionality of the organic matrix in magnetite biomineralization. These structural and mineralization mechanisms were compared with the biological samples. The comparative studies of the structural features will help to optimize the structure of materials for improved mechanical properties.

Keywords: Biomineralization, Magnetite, Gelatin, Hydrogels, SANS, VSANS, SAXS
# Table of Contents

Preamble ................................................................................................................................. iii  
Acknowledgment .................................................................................................................... v  
Zusammenfassung ................................................................................................................... vii  
Abstract ................................................................................................................................ ix  
Table of Contents .................................................................................................................. xi  
Abbreviations ....................................................................................................................... xvii  

## 1 General Introduction  
1.1 Biomineralization ........................................................................................................... 1  
1.1.1 Nacre—the Mother of Pearl ....................................................................................... 3  
1.1.2 The Tooth of Chiton .................................................................................................... 4  
1.1.3 Magnetotactic Bacteria ............................................................................................... 6  
1.2 Bio-inspired Mineralization ........................................................................................... 7  
1.3 Organic matrix .............................................................................................................. 9  
1.4 Aim of this thesis .......................................................................................................... 9  

## 2 Small Angle Scattering  
2.1 Basics of Small Angle Scattering ................................................................................ 13  
2.1.1 Scattering of X-rays and Neutrons ........................................................................... 14  
2.1.2 The Macroscopic Differential Scattering Cross Section ....................................... 15  
2.1.3 Scattering Length Density and Contrast ............................................................... 17  
2.2 Small Angle Neutron Scattering ................................................................................ 17  
2.2.1 The SANS Instrument ............................................................................................. 19  
2.2.2 Planning a SANS Experiment ................................................................................ 20  
2.3 Small Angle X-Ray Scattering .................................................................................... 22  
2.3.1 The SAXS instrument ............................................................................................ 24  
2.3.2 The SAXS experiment ............................................................................................ 25  
2.4 Analysis of Small-Angle Scattering Data .................................................................... 24  
2.4.1 Model Independent Analysis .................................................................................. 26  
2.4.2 Some Empirical Expressions ................................................................................... 27  
2.4.3 A Small-Angle Scattering Analysis of Hierarchical Structure .............................. 28  
2.4.4 Anisotropic Small Angle Scattering ...................................................................... 29  
2.4.5 Shape Reconstruction from SAS Data ...................................................................... 39  

- xi -
Table of Contents

2.5 Summary............................................................................................................. 42 -

3 Gelatin Hydrogel Matrices ............................................................................... 45 -
3.1 Introduction ........................................................................................................ 45 -
3.2 Experimental Section ....................................................................................... 47 -
3.2.1 Materials ........................................................................................................ 47 -
3.2.2 Synthesis of Gelatin Hydrogels .................................................................. 48 -
3.2.3 SANS and VSANS Experiments ................................................................. 48 -
3.2.4 SAXS Experiments ...................................................................................... 48 -
3.2.5 Thermal Analysis .......................................................................................... 49 -
3.2.6 Rheological Experiment .............................................................................. 49 -
3.2.7 Atomic Force Microscopy (AFM) ................................................................ 49 -
3.2.8 Powder X-ray Diffraction (XRD) ................................................................. 50 -
3.3 Results and Discussion .................................................................................... 50 -
3.3.1 General Macroscopic Properties of Gelatin Hydrogels ......................... 50 -
3.3.2 The Micro Structure of Gelatin Hydrogels ............................................... 54 -
3.3.3 The Structure as a Function of Gelatin Concentration .............................. 59 -
3.3.4 The Contrast in Gelatin Hydrogels ............................................................. 66 -
3.3.5 The Temperature Effects ............................................................................ 71 -
3.3.6 The pH Effects ............................................................................................. 76 -
3.3.7 Salt Effects .................................................................................................... 79 -
3.4 Summary ........................................................................................................... 82 -

4 Superparamagnetic Magnetite Mineralization in Gelatin Hydrogels ...... 85 -
4.1 Introduction ........................................................................................................ 85 -
4.1.1 Superparamagnetic Magnetite ................................................................... 85 -
4.1.2 Coprecipitation Methods ............................................................................ 85 -
4.1.3 Bio- and Bioinspired Mineralization of Magnetite .................................... 86 -
4.1.4 The Aim of This Part ................................................................................... 87 -
4.2 Experimental Section ...................................................................................... 88 -
4.2.1 Materials ....................................................................................................... 88 -
4.2.2 Synthesis of Gelatin Hydrogels ................................................................. 88 -
4.2.3 Magnetite Mineralization in Gel Matrices ............................................... 88 -
4.2.4 SANS and VSANS Experiments ................................................................. 89 -
4.2.5 SAXS Experiments ...................................................................................... 90 -
4.2.6 Atomic Force Microscopy (AFM) ............................................................... 90 -
4.2.7 Transmission Electron Microscopy (TEM) ............................................... 90 -
4.2.8 Powder X-ray diffraction (XRD) ................................................................. 91 -
4.2.9 Superconducting Quantum Interference Device (SQUID) ....................... 91 -
4.3 Theoretical Part .............................................................................................. 91 -

xii
4.3.1 Model for Small-Angle-Scattering .............................................................. - 91 -  
4.3.2 Molecular Simulation ....................................................................................... - 92 -  
4.4 Results and Discussion .................................................................................. - 93 -  
4.4.1 The Iron-loaded Gelatin Precursors .............................................................. - 93 -  
4.4.2 Magnetite Mineralization in the Iron-Loaded Gelatin Hydrogels .............. - 97 -  
4.4.3 The Iron Concentration Influence on the Mineralization ......................... - 105 -  
4.4.4 Mechanism of Magnetite Mineralization in the Hydrogels ....................... - 110 -  
4.4.5 Drying induced Reorganization in the Hybrid Materials ......................... - 113 -  
4.5 Conclusions .................................................................................................... - 119 -  

5 Formation of Large Magnetite Nanoparticles in Gelatin Hydrogels .......... - 123 -  
5.1 Introduction ..................................................................................................... - 123 -  
5.1.1 Stable Single Domain Magnetite Particles ............................................... - 123 -  
5.1.2 Synthesis of Stable Magnetite Single Domain Nanoparticles ................. - 124 -  
5.1.3 The Aim of this Part ..................................................................................... - 125 -  
5.2 Experimental Section ..................................................................................... - 126 -  
5.2.1 Materials .................................................................................................. - 126 -  
5.2.2 Synthesis of Gelatin Hydrogels ................................................................ - 126 -  
5.2.3 Magnetite Mineralization in Gel Matrices ............................................... - 126 -  
5.2.4 SANS and VSANS Experiments ............................................................... - 127 -  
5.2.5 Transmission Electron Microscopy (TEM) ............................................... - 129 -  
5.2.6 Powder X-ray diffraction (XRD) .............................................................. - 129 -  
5.2.7 Superconducting Quantum Interference Device (SQUID) ....................... - 129 -  
5.3 Results and Discussion .................................................................................. - 130 -  
5.3.1 The Iron(II)-loaded Gelatin Precursors ..................................................... - 130 -  
5.3.2 Large Magnetite Formation in the Hydrogels ............................................. - 131 -  
5.3.3 SANS Contrast Variation Studies of the Composites ............................... - 134 -  
5.3.4 The Influence of Oxidant and Iron Source Concentration ....................... - 137 -  
5.3.5 Large Nanoparticle Mineralization Kinetics .............................................. - 140 -  
5.4 Conclusion ..................................................................................................... - 144 -  

6 Two Step Magnetite Mineralization in Gelatin Hydrogels .................. - 147 -  
6.1 Introduction and Aim ..................................................................................... - 147 -  
6.2 Experimental Section..................................................................................... - 147 -  
6.2.1 Materials .................................................................................................. - 147 -  
6.2.2 The two-step Mineralization of Magnetite Nanoparticles ....................... - 148 -  
6.2.3 SANS and VSANS Experiments ............................................................... - 148 -  
6.2.4 Powder X-Ray Diffraction (XRD) .............................................................. - 149 -  
6.3 Results and Discussion .................................................................................. - 149 -  
6.4 Conclusion ..................................................................................................... - 152 -  

xiii
Table of Contents

7 Magnetite Mineralization in Gelatin Hydrogels in Nacre Organic Matrix ..........................................................-155-
  7.1 Introduction ..................................................................................................................................................-155-
  7.2 Experimental Section ...............................................................................................................................-156-
    7.2.1 Materials ..............................................................................................................................................-156-
    7.2.2 Preparation of insoluble organic nacre matrix .......................................................................................-157-
    7.2.3 Infiltration of gelatin inside the insoluble nacre matrix .....................................................................-157-
    7.2.4 Magnetite Formation in Gelatin-Nacre Organic Hybrid Matrix .........................................................-158-
    7.2.5 SANS and VSANS Experiments ........................................................................................................-158-
    7.2.6 SAXS Experiments .............................................................................................................................-160-
    7.2.7 Transmission Electron Microscopy (TEM) ..........................................................................................-161-
    7.2.8 Scanning Electron Microscopy (SEM) ..................................................................................................-161-
  7.3 Results and Discussion ..............................................................................................................................-161-
    7.3.1 The Hierarchical Structure of Nacre Organic Matrix .........................................................................-161-
    7.3.2 Gelatin Hydrogel inside of Nacre Organic Matrix .............................................................................-166-
    7.3.3 Magnetite formation in the Hybrid Organic Matrix ............................................................................-167-
  7.4 Conclusions ..................................................................................................................................................-168-

8 Summary and Outlook ..............................................................................................................................................-171-
  8.1 Summary ......................................................................................................................................................-171-
  8.2 Outlook ..........................................................................................................................................................-175-

Appendix A: General Parameters .............................................................................................................................-177-
  A.1 Radius of gyration .......................................................................................................................................-177-
  A.2 Scattering length density .............................................................................................................................-178-

Appendix B: The effect of water and heavy water influence on the mineralization ..............................................-179-
  B.1 Magnetite mineralization in H2O and D2O (Coprecipitation) ....................................................................-179-
  B.2 Magnetite formation in H2O and D2O in gelatin hydrogels ......................................................................-180-

Appendix C: SANS-VSANS Experiments .............................................................................................................-181-
  C.1 Small angle neutron scattering diffractometer, KWS-1 .........................................................................-181-
  C.2 Very small angle neutron scattering diffractometer, KWS-3 .................................................................-183-
  C.3 Sample Environment .....................................................................................................................................-186-

Appendix D: SAXS Experiments ............................................................................................................................-187-

Appendix E: Lists of Equations ............................................................................................................................-188-

List of Figures and Tables ........................................................................................................................................-194-
<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bibliography</td>
<td>209</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
</tr>
<tr>
<td>--------------</td>
<td>-------------</td>
</tr>
<tr>
<td>AFM</td>
<td>Atomic Force Microscopy</td>
</tr>
<tr>
<td>DSC</td>
<td>Differential Scanning Calorimetry</td>
</tr>
<tr>
<td>EB</td>
<td>Empty Beam</td>
</tr>
<tr>
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<td>Field-Cooled</td>
</tr>
<tr>
<td>FFT</td>
<td>Fourier Analysis</td>
</tr>
<tr>
<td>FWHM</td>
<td>Full Width At The Half Maximum</td>
</tr>
<tr>
<td>MLZ</td>
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<td>Paul Scherrer Institute</td>
</tr>
<tr>
<td>SD</td>
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</tr>
<tr>
<td>SEM</td>
<td>Scanning Electron Microscope</td>
</tr>
<tr>
<td>SLD</td>
<td>Scattering Length Density</td>
</tr>
<tr>
<td>SANS</td>
<td>Small Angle Neutron Scattering</td>
</tr>
<tr>
<td>SAS</td>
<td>Small Angle Scattering</td>
</tr>
<tr>
<td>SAXS</td>
<td>Small Angle X-Ray Scattering</td>
</tr>
<tr>
<td>SSD</td>
<td>Stable Single Domain</td>
</tr>
<tr>
<td>SQUID</td>
<td>Superconducting Quantum Interference Device</td>
</tr>
<tr>
<td>TB</td>
<td>Blocking Temperature</td>
</tr>
<tr>
<td>TGA</td>
<td>Thermogravimetric Analysis</td>
</tr>
<tr>
<td>TEM</td>
<td>Transmission Electron Microscopy</td>
</tr>
<tr>
<td>USAS</td>
<td>Ultra Small Angle Scattering</td>
</tr>
<tr>
<td>VSAS</td>
<td>Very Small Angle Scattering</td>
</tr>
<tr>
<td>VSANS</td>
<td>Very Small Angle Neutron Scattering</td>
</tr>
<tr>
<td>WAXD</td>
<td>Wide-angle X-ray Diffraction</td>
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<td>XRD</td>
<td>Powder X-ray Diffraction</td>
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<td>ZFC</td>
<td>Zero-Field-Cooled</td>
</tr>
</tbody>
</table>
1 General Introduction

1.1 Biomineralization

Biomineralization refers to the processes by which living organisms produce minerals\(^1,2\) in the form of skeletons,\(^3\) molluscan shells,\(^4\) diatoms,\(^5\) teeth\(^6-9\) and bones\(^10-14\) (Figure 1.1). It is a widespread biological phenomenon that occurs in almost all organisms from prokaryotes (e.g., magnetite nanocrystals in certain bacteria\(^15\)) to humans (bone and teeth\(^16\)). These biologically produced biominerals are inorganic/organic hybrid composites formed under conditions of moderate temperature, pressure and pH showing remarkable materials properties, controlled hierarchical structures which has received much attention because they are considered natural archetypes for future materials.\(^17-24\)

It is known that in the composites the small amounts of organic component are essential for the formation of biominerals.\(^25\) These biomacromolecules not only significantly improve the mechanical properties of the resulting composites but also exert a crucial control on the biomineralization process, contributing to the determination of the crystal size, polymorphs, morphology and crystallographic orientation.\(^1,26\) Therefore, biological routes of structuring biominerals are becoming valuable approaches for practical engineering processes.

In nature, over 400 millions of years organisms have evolved strategies to produce mineralized structures, each with unique composition, morphology, and mechanical properties.\(^27\) Now it is known that more than 60 different types of biominerals are found, specially tailored for a wide variety of functions.\(^1\) These biominerals are formed under well-controlled conditions which are highly optimized materials and abundant existing in nature. In many cases, a biomineral serves as mechanical support and protection of soft tissues, such as with the shells of mollusks,\(^28\) or as a defense, such as with the spines of sea urchins,\(^29\) or as support, for example skeletons.\(^30\) Other special uses that living organisms give to biominerals are: feeding tools, such as teeth,\(^9\) or magnetic sensors in magnetotactic bacteria (magnetite),\(^31\) gravity sensing devices (CaCO\(_3\)) and iron storage (Fe\(_2\)O\(_3\)-H\(_2\)O in ferritin).\(^32\) About 80 % of the biominerals are crystalline and 20 % of the biominerals are amorphous among the known cases, where calcium containing
General Introduction

minerals represent a half of all known biominerals and are abundant both in the oceans and on land. Other examples include silica, found for example in plants, diatoms and sponges and iron oxides, present in bacteria, mollusks, and plants.\(^1\)

![Figure 1.1. Hierarchical structures of tough biominerals.](image)

(A) Lamellar micro-architectures of Nacre, mineral bridges between mineral tiles, the fibrous chitin network that forms the organic matrix.\(^{26, 34}\)

(B) Lobster exoskeleton showing the twisted plywood structure of the chitin and the tubules that extend from the chitin layers to the animal.\(^{35}\)

(C) Antler bone image showing the hard outer sheath (cortical bone) surrounding the porous bone. The collagen fibrils are highly aligned in the growth direction, with nano-crystalline minerals dispersed in and around them.\(^{36}\)

(D) Silica sponge and the intricate scaffold of spicules. Each spicule is a circumferentially layered rod: The interfaces between the layers assist in arresting crack propagation.\(^{37}\)

The mechanisms of biomineralization are not fully understood. The biomineralization processes can be divided into two fundamentally different groups based on their degree of biological control: the biologically induced and organic matrix-mediated biomineralization.\(^1, 38\) In both biologically induced and mediated biomineralization, the organic components (organic matrix and nucleation protein) are formed first, and then these bind ions, which serve as nucleation sites for crystal growth. Next self-assembly and epitaxial crystal growth subsequently complete the composite structure. Study on the
biomineralization mechanism may provide models for new materials concepts, inspire design solutions and give new insights into the genetic control of the biological structure.

Here three different examples of mineral formation in biological systems are introduced: nacre, tooth of chiton and magnetotactic bacteria.

### 1.1.1 Nacre-the Mother of Pearl

Nacre, known as the mother of pearl, is the iridescent inner surface of some mollusk shells, which consists of highly oriented aragonitic crystals and an organic matrix (polysaccharides and proteins).³⁹,⁴⁰ It is well known for its beautiful iridescence but also for the outstanding mechanical properties e.g. high fracture toughness. This organic/inorganic hybrid structure makes nacre 3000 times more fracture resistant as compared to aragonite which makes up ca. 95 wt.-% of this structure.⁴¹

![Diagram of nacre structure](image)

**Figure 1.2.** The hierarchical structure of nacre.⁴² (A) A sketch diagram showing the construction of nacre from a chitin molecule to a shell in bivalves. (B) Inside view of the shell. (C) A fractured transverse section. (D) SEM showing the morphology of mineralization of bivalves (top view). (E) Cryo-TEM image showing the homogenous texture and layered structure. (F) A sketch showing mineral growth through the mineral bridges. (H) Nonmineralized nacre layer matrix.⁴⁰

Nacre has a hierarchical microarchitecture that spans over multiple length scales from nanoscale to macroscale. The basic structure can be described by a brick-and-mortar model, in which the hard hexagonal aragonite platelets (0.2-0.5 μm in thickness and 5-15 μm in diameter) are glued together with soft organic materials (chitin surrounded by...
acidic proteins)\textsuperscript{43} (see Figure 1.2). Lamellar micro-architectures of hard aragonite bricks with soft organic layers in between can be thought of as glue mortar (Figure 1.2 C), which causes crack deflection and resists slip in order to provide toughness and impact resistance. The aragonite platelets are strongly oriented, such that the (001) plane of aragonite is parallel to the plane of the interlamellar organic matrix.\textsuperscript{44, 45}

The process of the biomineralization by which the nacre forms involves the selective identification and uptake of inorganic elements from the local environment and their incorporation into structures under strict biological control.\textsuperscript{45} The growth of the biomineral is regulated by a small amount of organic material (Maximum 5\% w/w, polysaccharides and proteins, see Figure 1.2 H), which exerts complete control over nucleation, polymorph selection, and morphology.\textsuperscript{46, 47} Nacre platelets begin to grow within the intermediate spaces, first quickly along the vertical direction (along the C-axis), at the same time as the inter-lamellar membranes separate to the required distance to accommodate the final thickness of the aragonite plates.\textsuperscript{46} The platelets later expand sideward until impinging on each other, so as to fill all of the available space\textsuperscript{46}(Figure 1.2 F). The shell thickens vertically and enlarges (horizontally) as the animal grows.\textsuperscript{1, 45}

1.1.2 The Tooth of a Chiton

Chitons are an ancient group of mollusks with a fossil record of more than 400 million years, which are classified as class Polyplacophora.\textsuperscript{48} They can be recognized by their eight overlapping shell plates mineralized by aragonite, which are firmly anchored in a tough muscular girdle. Chitons scratch algae from the rocks by using of a radula, which require wear resistant teeth. The radula (Figure 1.3) has been coined as a conveyor belt of continuously developing teeth, replaced by new teeth as they are worn out. In nature, the teeth are one of the amazing iron contained biominerals which are actually the hardest known biomineral. They are hardened by the inclusion of magnetite nanoparticles (15-20 nm) into a protein-polysaccharide gel matrix.\textsuperscript{49} The teeth usually consist of a hard shell/soft core structure.\textsuperscript{50} The outer shell consists of magnetite while the core is mainly made of calcium apatite (Figure 1.3 E). Lepidocrocite and goethite are found at the thin layer between the core and shell.\textsuperscript{2, 51} The remarkable functional properties of the resulting mineral composites, such as outstanding hardness and wear resistance, can be attributed to the buried organic–inorganic interfaces at multiple hierarchical levels and the highly mineralized inorganic content (ca. 70 wt. %).
Figure 1.3. Representative images of the chiton radula, with a demonstration of: (A) Chiton magnificus Deshayes, from Chile. (B) Chiton radula and magnet. (C) Light microscopy image of chiton radula. (D) Light microscopy image of the chiton tooth, the cross section. (E) EDX mapping results on a cross section of the chiton tooth. (F) AFM phase image of the tooth magnetite shell.

As the old teeth discarded, within a couple of days new magnetite teeth move into place having been produced at the other end of the radula.\(^1\) It is an exquisite example of nature at its best, where matrix-mediated biomineralization controls the deposition of a wide range of minerals in architecturally discrete regions resulting in highly specialized feeding implements. For the teeth mineralization, the cells of the Chiton first create the housing of a mature tooth, then the framework of α-chitin, proteins on which iron hydroxides precipitate.\(^2\) Then the iron protein, ferritin is brought to the tooth location and stored until iron is extracted and transported in a reduced, soluble form to the mineralizing sites.\(^2,9,50\) Mineralization of the inorganic component initiated via the deposition of ferrihydrite aggregates at the leading edge of the tooth tips. These ferrihydrite aggregates subsequently transform to magnetite. The aggregates further form highly oriented rod-like particles that exhibit regionally defined geometries depending on the periodic spacing of the surrounding chitin fiber like matrix.\(^48,49,52\) Finally the deposition
of the biominerals within an organic framework facilitates intricate crystallographic design and structure, and imparts unique properties to the chiton teeth, such as tensile strength, shock absorption, controlled wear and abrasion.

1.1.3 Magnetotactic Bacteria

Magnetotactic bacteria (MTB) are a polyphyletic group of microorganisms with the ability to orient and migrate along geomagnetic field lines.\(^1\),\(^3\),\(^1\),\(^3\),\(^1\) This ability is based on specific intracellular structures, the magnetosomes, in which are specialized organelles synthesized by MTB for geomagnetic navigation in their aquatic habitats.\(^5\) The magnetosomes comprise enveloped membrane, nano-sized crystals of magnetite (Fe\(_3\)O\(_4\)) or the greigite (Fe\(_3\)S\(_4\)).\(^5\),\(^6\) They are arranged in intracellular chains (Figure 1.4 A) that enable the bacteria to align and swim along earth magnetic fields, known as “magnetotaxis”.\(^5\) The magnetic particles ranging from 30 to 120 nm in diameter are well crystallized with distinctive single crystal morphology, and several crystallites appear within one bacterium and become aligned.\(^5\),\(^6\)

![Figure 1.4. Representative images of Magnetotactic bacteria: (A) TEM image of a magnetotactic bacterium. Note the chain of twelve magnetite (Fe\(_3\)O\(_4\)) nanoparticles that are arranged along the long axis of the cell.\(^5\) (B) Model of the iron reaction pathway, and roles of the proteins that were so far shown to be necessary for magnetite biomineralization and chain formation.\(^5\)](image-url)
The mineralization process by MTB within bacteria is still not well understood. However, people believe several distinct steps are involved in this biomineralization process (Figure 1.4 B). These steps include: (I) iron uptake by the bacterial cell, magnetosome vesicle formation within the bacteria, (II) iron transport into the magnetosome vesicle, and (III) protein-mediated magnetite biomineralization. In the above third step, first a precursor iron oxide forms that matures into a single magnetic domain crystal of a magnetic mineral. The linear arrangement of the crystallites into a chain of magnetic particles, orient so as to enhance the magnetic dipole moment of the bacterium. MTB swim up and down using their geomagnetic navigation to find optimum oxygen concentration locations, and probably move when the oxygen concentration changes.\textsuperscript{2, 57, 60} The biological control of internal mineral formation and the choice of the mineral species allow the bacteria to use both magnetotaxis and aerotaxis to maximize their habitat.\textsuperscript{57}

### 1.2 Bio-inspired Mineralization

Natural biological materials are built at mild conditions via a sophisticated process from a fairly limited selection of components. From nacre to bone, these biominerals show unique structural and functional properties through the combination of hard inorganic and soft organic phases in complex hierarchical structures, with characteristic dimensions spanning from the nanoscale to the macroscale.\textsuperscript{10, 17, 27, 61, 62} The resulting materials are usually lower density and display unique combinations of strength and toughness, and other remarkable features. These natural composites are often created through sophisticated biomineralization processes that result in an accurate control of the shape, size, and distribution of the inorganic crystals. Thus, nature is indeed a school for materials science and its associated disciplines such as chemistry, biology, physics or engineering. Learning from nature in the laboratory to build new bio-inspired composites is a very attractive prospect.\textsuperscript{62}

In recent years, scientists increasingly use biominerals as an inspiration for new biomimetic materials.\textsuperscript{11, 19, 47, 63, 64} For example, Finnemore et al\textsuperscript{65} present a route to artificial nacre which mimics the natural layer-by-layer approach to fabricate a hierarchical crystalline multilayer material (Figure 1.5). They report the route to a nacre-like CaCO\textsubscript{3} multilayer that includes a minimum of essential stages outlined as follows: (1) Stabilisation of ACC in solution, (2) Specific aggregation and continuous film
formation on organic surfaces, (3) Deposition of a porous, suitably functionalised thin organic film on a previously formed mineral layer, (4) Crystallisation of the formed ACC layers to aragonite or calcite, and (5) Cyclical iteration of steps 1-4. By mimicking the steps in a cyclical deposition protocol, an organic/calcite multilayer stack that approximates natural nacre was formed. This route resulted in a 5-35 µm-sized polycrystalline colored structure organized in 400 nm-thick plates that are interconnected through porous organic films. The hybrid structure gives rise to nacre-like enhanced toughness. The good control over the layer periodicity reproduces nacre’s iridescence. Both the growth strategy and final material bear a close resemblance to natural nacre.

Figure 1.5. Comparison of natural and artificial nacre.65 a, Image showing natural nacre’s bright iridescence (scale bar 5 mm). b, SEM image of a stack of mineral platelets on the fractured surface (scale bar 2 µm). c, Organic inter-crystalline film that allows for vertical crystal continuity between platelets (scale bar 500 nm). d, Artificial Nacre, exhibiting a similar coloration as in a (scale bar 5 mm). e, SEM image of the fractured surface showing 7 aligned CaCO3 platelets separated by organic films. The surface graininess is comparable to natural nacre (scale bar 1 µm). f, SEM image of PVP film on calcite showing a similar pore distribution as in c (scale bar 300 nm). g, AFM height image of the porous film (scale bar 300 nm).

Finally, the study of biomineralization has influenced more and more scientists interested in controlling hierarchical materials synthesis from molecular to macroscopic levels. The understanding of biology controlled mineral growth processes and marrying the structural control found in nature with the huge variety of synthetic compounds, as well as developing new materials, extending the range of application of current ones, and breaking existing limitations in terms of mechanical properties, will catalyze new enthusiasm for biomimetic approaches to materials manufacture64,66.
1.3 Organic matrix

The organic matrix plays important roles in controlling mineral growth during the bio/bio-inspired mineralization including the control over mineral phases formed, shape of the mineral particles, and their organization. The structural organization and the constituents of the organic matrix are essential to control the mineralization processes. Those organic matrices in many live organisms appear to be organized by a core of relatively hydrophobic structural macromolecules (proteins and polysaccharides) and surface layers of acidic proteins/polysaccharides. In many cases of biominerals, the conformations and orientations of matrix constituents are in relation to the mineral crystal lattice. The major matrix constituents are aligned with one or more mineral crystallo- graphic axes. These observations suggest that the organic matrix plays specific roles in mineralization. A typical example of biominerals exists in nature and the organic matrix is the human bone, where the biominerals are composed with stiffer apatite mineral and soft fibrillar collagen type I and acidic proteins. Fibrillar collagen comprises close to 90% of its total organic content with other acidic proteins macromolecules which account for the remaining 10%. Hydroxyapatite crystals are arranged such that the c axis of crystal aligns with the fibril axis.

1.4 Aim of this thesis

Inspired by these biomaterials design concepts, the motivation of the project is to develop biomimetic composite structures which combine the best of the properties of three different biominerals by structural design: The fracture toughness of nacre, the wear resistance of chiton teeth and the magnetic properties of co-aligned magnetite nanoparticles in magnetotactic bacteria. In detail for the purpose to mimic the chiton tooth structure, magnetite nanoparticles are first synthesized inside the gelatin hydrogels in order to form a highly mineralized organic-inorganic hybrid body. By variation in the synthesis protocol, the control of the magnetite nanoparticle size to be superparamagnetic (< 30 nm) for inducible magnetic dipoles or ferrimagnetic (> 30 nm) for fixed magnetic dipoles can be achieved. Second, in order to improve the diffusion of iron ions in the hydrogels and the uniformity of the final composites, we introduce the nacre organic matrix (see Figure 1.6). By replacing a bulk gel with a thin-layered structure, the gel slice gets thinner so diffusion is enhanced. The application of a demineralized nacre
matrix, to act as structural template was suggested to successfully format the nacre tablet structure in the hybrid materials. Furthermore, magnetite mineralization in a magnetic field can imprint magnetic dipole orientation for ferrimagnetic nanoparticles adding highly anisotropic magnetic properties to the hybrid material on top of the envisaged beneficial mechanical properties.

In the above-proposed materials, there are multiple components which include both organic matrix and inorganic minerals. Especially the organic components are soft and sensitive to the environment and easily damaged during some characterization process.

![Figure 1.6. Schematic representation of the materials synthetic concept. (A) Magnetite formation in a thin gelatin hydrogel 2D films. (B) Magnetite formation in thin gelatin hydrogels in the nacre organic matrix (Cellular Fractal Ferro Gel). (C) Magnetite formation in bulk gelatin hydrogels (the core and the surface mineralization may be very different) (3D Bulk Gel).](image)

Small-angle Neutron / X-ray Scattering (SANS/SAXS) is an important non-destructive technique for the determination of structural properties in materials science and engineering and has to be considered as a complementary tool of transmission electron microscopy (TEM). It is successfully applied for the characterization of the microscopic structure in solid condensed materials as well as in soft matter science for studying the conformation of polymers as well as the phase behavior of microemulsions, liquid crystals, and proteins. An important advantage of SANS is the possibility of contrast variation by the exchange of H2O and D2O concentration in the aqueous solution or as in the...
case of a magnetite phase the separation of nuclear and magnetic scattering. Both techniques of contrast variation were used in this thesis in order to analyze the individual phases besides their structural dimensions also with respect to their coherent scattering length density determined by the composition and mass density.

Thus, inspired by the above three biomineralization examples, the **aim of this work** is to investigate the mechanism of bio-inspired magnetite mineralization in an organic matrix (gelatin hydrogels and nacre organic matrix) and the organic-inorganic hybrid structures by Small Angle Neutron / X-ray Scattering methods. The expected results will provide important information for better understanding and prediction of bio-inspired magnetite mineralization. This will help us to improve our bio-inspired organic-inorganic hybrid materials synthesis.

In order to reach the aim, this thesis is organized in eight main chapters. The first Chapter shows a brief introduction to the knowledge of biomineralization, the materials synthetic concept and the aim of this thesis. The second Chapter gives a brief description of the Small Angle Scattering method with the principle, instrument design, and the data analysis method. The third part focuses on the gelatin hydrogels, which is the main material used in this project as biomineralization media. From the fourth chapter to the seventh chapter, the work is focused on the magnetite mineralization in organic matrices as well as investigation on the mineralization mechanisms by using small angle scattering methods. Chapter 4 presents the small magnetite nanoparticles formation in the hydrogels via a co-precipitation method. Chapter 5 describes the method of partial oxidation of large magnetite particles. Chapter 6 combines the two recipes of magnetite mineralization in two-step strategies. Chapter 7 introduces two organic matrix hybrids as media for magnetite mineralization to form layered magnetic gel composites: demineralized nacre organic matrix filled with gelatin gel is aimed to producing the layered structure. Finally, in Chapter 8, conclusions drawn from all of the results are discussed.
2 Small Angle Scattering

Small-angle scattering (SAS) of x-rays (SAXS) and neutrons (SANS) is a fundamental method where the elastic scattering of x-rays/neutrons by a sample which has inhomogeneities in the nm-range (usually from 1 nm to 300 nm, see Figure 2.1), is recorded at very low angles (<10°).69-72 The x-ray photons / neutrons interact with the particles of a sample and are scattered. The detected scattering pattern is characteristic of the nanoscale structures and can be used to determine their size, shape, distribution, internal structure, orientation and more.

As compared to direct imaging techniques such as TEM (transmission electron microscopy), SAS has several advantages71,73: 1) SAS is a non-destructive method for investigating nanostructures in liquids and solids. 2) SAS allows one to study the structure of native particles in close to physiological environments and to probe structural changes in response to variations in external conditions, such as temperature, magnetic field, pressure. 3) Due to the averaging over a large sample volume, SAS provides structural parameters with a high statistical accuracy. 4) It is possible to perform time-resolved SAS measurements in combination with Raman spectroscopy, IR, Rheometer and other in-situ techniques to explore the kinetics. 5) Not only the disordered structure, but also the structure of ordered systems like lamellae, and fractal-like materials can be studied. In addition, combination with ultra-small-angle scattering (USAS), the structure can be resolved for even larger dimensions up to several µm. However, since SAS is an indirect technique, the combination with direct imaging techniques is often very useful to develop an accurate way of interpreting the scattering data. It is successfully applied for the characterization of the microscopic structure in solid condensed materials (metals and alloys, ceramics) as well as in soft matter science for studying the conformation of polymers as well as the phase behavior of microemulsions, liquid crystals, colloids and proteins (Figure 2.1). SAS also made it possible to investigate intermolecular interactions including assembly and conformational changes in real time, on which biological function often relies.
2.1 Basics of Small Angle Scattering

The methods of small-angle scattering are based upon the analysis that elastic scattering occurs between the incoming wave and a particle, causing the reflected waves to scatter in all directions. In order to compare results obtained at different wavelengths, detector distance, and scattering angles or with different scattering beam, a more convenient parameter is the scattering vector $Q$, as defined in Figure 2.2. The scattering vector $Q$ is defined as the difference between the wave-vector of the incoming $k_i$ and

![Figure 2.1. Size range comparisons from micro to macro scale and the SAS applied range.](image-url)
outgoing beam \( k_s \), where \( \lambda \) is the wavelength of the x-ray photons or neutrons. This quantity indicates the typical length scales probed by the scattering experiment, which has dimensions of \((\text{length})^{-1}\) normally quoted in \(\text{nm}^{-1}\) or \(\text{Å}^{-1}\). Combining SAS and USAS, the Q-range covered can be three orders of magnitude, typically \(0.0001 \text{ Å}^{-1} < Q < 0.6 \text{ Å}^{-1}\) corresponding to a real space dimension of \(6 \upmu\text{m}\) down to \(1 \text{ nm}\).

**Figure 2.2.** Definition of scattering vector \( Q \), with the scattering angle \(2\theta\), the incoming beam \( k_i \) and scattered beam \( k_s \).

### 2.1.1 Scattering of X-rays and Neutrons

X-ray photons with an energy \( E \) have a wavelength \( \lambda = 12.56/E \), where \( \lambda \) is expressed in Å and \( E \) in keV. For SAXS, relatively hard x-rays with energies around 10 keV are used (\( \lambda \) about 1.0-1.5 Å).\(^{71}\) The neutron wavelength is given by de Broglie’s relationship, \( \lambda [\text{Å}] = 3966/v [\text{ms}^{-1}] \), here \( v \) is the velocity of neutrons, and neutrons with wavelengths \( \lambda \) from 2 - 20 Å are typically used.\(^{71,75}\) Thus, a neutron with a wavelength of 1.5 Å has an energy of 36.4 meV. By contrast, the energy of a 1.5 Å X-ray photon is \(~8.2 \text{ keV} \), more than 200,000 times greater than the energy of the neutron.\(^{70-72,76}\) Neutrons, therefore, have a particular advantage over X-rays in the study of sensitive samples, such as biological material for example.

Although the physical mechanisms of elastic x-ray photons and neutron scattering by matter are different, they share the similar physical formalism. The most fundamental difference between x-ray and neutron is the mechanism by which the incident radiation interacts with matter. X-rays photons are scattered by the electrons surrounding atomic
nuclei, but neutrons are scattered by the nucleus itself. This single fact has several important consequences.

Table 2.1. Comparison of the (coherent) scattering length for neutrons and x-rays for a selection of elements. The area of the colored circles represents the scattering length. All of this data was taken from the Special Features section of neutron scattering lengths and cross sections of the elements and their isotopes in Neutron News.

<table>
<thead>
<tr>
<th>Element</th>
<th>$b_{\text{neutrons}} \times 10^{12}$ cm</th>
<th>$b_{\text{x-rays}} \times 10^{12}$ cm</th>
<th>Atomic number Z</th>
</tr>
</thead>
<tbody>
<tr>
<td>$^1$H</td>
<td>-0.374</td>
<td>0.282</td>
<td>1</td>
</tr>
<tr>
<td>$^2$D</td>
<td>0.667</td>
<td>0.282</td>
<td>1</td>
</tr>
<tr>
<td>C</td>
<td>0.665</td>
<td>1.67</td>
<td>6</td>
</tr>
<tr>
<td>N</td>
<td>0.940</td>
<td>1.97</td>
<td>7</td>
</tr>
<tr>
<td>O</td>
<td>0.580</td>
<td>2.25</td>
<td>8</td>
</tr>
<tr>
<td>S</td>
<td>0.280</td>
<td>4.51</td>
<td>16</td>
</tr>
<tr>
<td>Fe</td>
<td>0.945</td>
<td>7.33</td>
<td>26</td>
</tr>
</tbody>
</table>

For X-rays scattered by a single atom, photons are primarily scattered by the electrons. The scattering intensity depends on the number of electrons (the atomic number $Z$). For X-rays scattered in the matter, the scattering length density for X-rays is proportional to the electron density. In contrast to X-rays, neutrons are scattered by the atomic nuclei and are sensitive to magnetic spin. The so-called scattering length $b$ describes the strength with which an atom scatters neutrons. The value for $b$ depends on the element, isotope and also the spin of the nucleus. Comprehensive lists of isotope neutron scattering lengths involved in this thesis are given in Table 2.1. The $b$ can have positive or negative values. It does not depend in a simple fashion on the atomic number, which allows detection of light elements, particularly hydrogen, in materials that contain heavy elements. This feature makes neutron complementary to x-ray scattering because x-ray scattering intensities are proportional to the square of the electron density. The interaction of neutrons with matter is weak and the absorption by most materials is correspondingly small. A neutron beam is therefore very penetrating. Neutrons, on the other hand can be used to probe the bulk properties of samples with path lengths of several centimeters or, alternatively, samples contained inside a complex apparatus (cryostats, furnaces, pressure cells etc). In addition, the neutron has a small magnetic moment which
can interact with the spin and orbital magnetic moments present in a sample containing atoms with unpaired electrons.\cite{70, 72, 75, 79, 81}

### 2.1.2 The Macroscopic Differential Scattering Cross Section

The scattering cross section is a measure of how “big” the electrons/nucleus appears to the photons/neutron and thus how strongly photons/neutrons will be scattered from it. The scattering from a macroscopic sample reflects both the total scattering intensity from all of the molecules and possible interference effects from waves scattered from different molecules.\cite{71} The interaction between the incident photons/neutrons and the scattering medium within the sample is contained in the quantity namely the differential scattering cross section (d\(\sigma/d\Omega\)).\cite{70, 72, 75} The incident photon/neutron flux per unit area per second (\(I_0\)) is scattered by a sample and the scattered photons/neutrons are acquired by each detector element subtending a solid angle \(\Delta\Omega\) with a detector efficiency \(\varepsilon\). The measured scattered intensity \(I_s\) is given by\cite{75, 82}:

\[
I_s = I_0 \varepsilon T_s \Delta\Omega A_s d \frac{d\Sigma}{d\Omega}
\]

(2.1)

where \(T_s\) is the sample transmission, \(A_s\) is the cross section of the beam, \(d\) is the sample thickness and \(d\Sigma/d\Omega\) is the differential scattering cross section per unit volume. The sample transmission can be calculated as \(T_s = I_T/I_0\), where \(I_T\) is the transmitted intensity per unit area per second. The quantity that can be directly compared to a model is \(d\Sigma/d\Omega(Q)\) which contains information about the structure and the interactions in the system over the range of \(Q\) spanned by the scattering experiment, and it is expressed in units of the reciprocal of length (usually in cm\(^{-1}\) or mm\(^{-1}\)). The \(d\Sigma/d\Omega(Q)\) is also called the “scattering intensity in absolute units”.\cite{78} Therefore, an essential step to reach a quantitative understanding of the measured intensities is the normalization of the experimental data to \(d\Sigma/d\Omega\) which can be denoted by \(I(Q)\) and is given simply in units of reciprocal length.\cite{71, 72, 75, 80, 82}

### 2.1.3 Scattering Length Density and Contrast

In order to describe the relation of macro material properties to the atomic properties, a quantity called the scattering length density (SLD) as a function of position in the sample was defined as\cite{71, 75, 79, 83}.\pagebreak
\[ \rho(r) = \frac{\sum_i^n b_i}{V} (r) \]  

(2.2)

where \( \rho \) is the scattering length density, \( b_i \) is the scattering length of the relevant atom and \( V \) is the volume containing the \( n \) atoms. Trying to connect the materials properties to the atomic properties, we can make the replacement of the sum in \(^{72, 75, 83}\)

\[ \frac{d\sigma}{d\Omega}(Q) = \frac{1}{N} \left| \sum_i^N b_i e^{iQ \cdot r} \right|^2 \]  

(2.3)

by the integral of the scattering length density distribution across the whole sample and normalized by the sample volume \(^{72, 75, 83}\)

\[ \frac{d\Sigma}{d\Omega}(Q) = \frac{N}{V} \frac{d\sigma}{d\Omega}(Q) = \frac{1}{V} \left| \int_V \rho(r) e^{iQ \cdot r} dr \right|^2 \]  

(2.4)

This equation is known as the “Rayleigh-Gans Equation” and shows us that SAS arises as a result of inhomogeneities in scattering length density. \(^{72, 75, 78, 83}\) The integral of the macroscopic cross section is the Fourier transform of the scattering length density distribution and the differential cross section is proportional to the square of its amplitude.

In the case of a system containing two phases with scattering length densities \( \rho_1 \) and \( \rho_2 \),

\[ |\Delta \rho|^2 = |\rho_1 - \rho_2|^2 \]  

is called the scattering contrast. Taking the equation 2.4 and breaking the total volume into two sub-volumes, \( V_1 \) and \( V_2 \). At non-zero \( Q \) values

\[ \frac{d\Sigma}{d\Omega}(Q) = \frac{1}{V} (\rho_1 - \rho_2)^2 \left| \int_{V_1} e^{iQ \cdot r} d\eta \right|^2 = \frac{1}{V} (\rho_1 - \rho_2)^2 \left| \int_{V_2} e^{iQ \cdot r} d\eta \right|^2 \]  

(2.5)

where scattering on a two-phase system the \( d\Sigma/d\Omega(Q) \) is proportional to the contrast. \(^{72, 75, 78, 83}\) From the equation 2.5, exchanging phase 1 for phase 2 gives the same scattering pattern.

In order to study the multi-component system, the ability to vary the scattering length density through contrast variation (such as the hydrogen-deuterium exchange) is a key
Small Angle Scattering

advantage of neutron scattering over other scattering techniques (x-rays, light).\(^7\) Figure 2.3 displays an ideal example of a core-shell type particle where contrast variation (color) can be used to highlight various parts of the particle structure. Thus the contrast variation method allows to “remove” scattering from parts of an object. The resulting scattering curves can be fitted simultaneously to the same model varying only the SLD.

![Figure 2.3](image.png)

**Figure 2.3.** A schematic representation of the effect of contrast variation on the measurable structure of a core-shell particle.

### 2.2 Small Angle Neutron Scattering

Small-angle neutron scattering (SANS) is an important non-destructive technique for the determination of structural length scales from 1 nm to several 100 nm in materials science and engineering and has to be considered as a complementary tool of electron microscopy. It has been ever-growing since its inception 40 years ago.\(^7\) SANS has been a major characterization method in research areas such as polymers\(^8\), complex fluids\(^9\), biology\(^7, 8\), nanoparticles\(^8\), macromolecular self-assemblies\(^9\) and materials science\(^9\). In addition, SANS can probe the internal structure of materials in situ, such as samples in solution, at elevated temperatures, under pressure, applied load or in the presence of an external magnetic field.\(^7, 8\) SANS has particular advantages over SAXS, having a larger penetration depth (up to several cm) and not suffering from issues of sample beam damage. Furthermore, using SANS contrast variation method, individual phases can be selectively highlighted via isotopic labeling or exchange of deuterium solvents in the aqueous solution. These latter benefits are particularly applicable to hydrogen-rich organic or biological materials\(^7\). Furthermore, the sensitivity to mag-
netism in which the neutron spin processes upon scattering allows both the magnitude and orientation of sample magnetic moments to be precisely determined. Additionally, the sensitivity to magnetism makes small angle neutron scattering ideal for the study of both the magnitude and orientation of sample magnetic moments.

This part is a focus on the use of a normal SANS instrument and reduces the raw 2D SANS data to 1D normalized data. The complementary use of Very Small Angle Neutron Scattering (VSANS) for an extension to a very small Q range (up to $10^{-4}$ Å$^{-1}$) is also explored.

### 2.2.1 The SANS Instrument

SANS and VSANS experiments were mainly carried out at the KWS1 and KWS3 diffractometers operated by Jülich Center for Neutron Research (JCNS) at the Heinz Maier-Leibnitz Zentrum (MLZ) in Garching, Germany. Some of the SANS data at large Q range is based on experiments performed at the SANS II, Swiss spallation neutron source SINQ, Paul Scherrer Institute, Villigen, Switzerland.

In the first case, MLZ neutron source is a steady-state reactor in which neutrons are continuously produced by fission processes. In the second case, the neutron source SINQ is a spallation source in which a pulsed neutron beam is generated by the collision of high-energy protons which chop off heavy atoms. The spectrometers KWS1 and KWS3 will be described as examples for steady-state and time-of-flight instrument respectively.

![Figure 2.4](image-url). A schematic representation of the KWS-1 SANS diffractometer: (1) S-shaped neutron guide NL3b; (2) high-speed chopper ($\approx 1-10\%$); (3) polarizer changer; (4) radio-frequency spin flipper; (5) beamstop.
neutron guide sections (18 × 1 m); (6) MgF₂ focusing lenses; (7) sample position with hexapod for heavy loading; (8) \(^{3}\)He analyzer with reversible polarization (to be implemented); (9) Anger-type scintillation detector.\(^{91}\)

The principle layout of a conventional pinhole SANS instrument KWS1 is shown in Figure 2.4: a monochromatic neutron beam with neutron wavelength \(\lambda\) (range 4.5 Å to 20 Å) is selected by a mechanical velocity selector from the continuous cold neutron spectrum delivered by the neutron source. In the case of SANS, the high-intensity requests can only be achieved by using an entire wavelength band, which for KWS1 is typically \(\Delta \lambda / \lambda = 10\). The divergence, size, and intensity of the beam sent to the sample is determined by an adaptive collimation system which consists of a set of mobile apertures allowing for the variation of the distance between the “entrance source” aperture and the “sample” aperture over a large interval (between 1m and 20 m). The scattered neutrons within a wide angular range, typically between 0.1° and 20°, are detected by varying the sample-to-detector distance between 1m and 20m. A large area position-sensitive detector with a space resolution of about 0.5÷0.8 cm can detect about 90-95% of the scattered neutrons due to using a beam-stop made of a neutron-absorbing material (Cd). An experimental data acquisition consists of in-house-made detector electronics and control software with a user-friendly interface. The covered Q range of the instrument extends from 0.0007 to 0.5 Å\(^{-1}\), which corresponds to sizes of features in the samples from 10 to 9000 Å.\(^{91,93}\)

---

**Figure 2.5.** A schematic representation of the KWS-3 VSANS diffractometer: (1) Neutron guide NL3a; (2) Velocity selector; (3) Entrance aperture; (4) Toroidal mirror; (5) Mirror chamber; (6) Sample positions 1 (10 m) and 2 (1 m); (7) Detector.\(^{94}\)

KWS-3 is a very small angle neutron scattering instrument running with a focusing mirror optical system (see Figure 2.5). The principle of this instrument is a one-to-one image of an entrance aperture onto a 2D position sensitive detector by neutron reflection from a double-focussing toroidal mirror. The instrument’s standard configuration with a 9.5 m sample-to-detector distance (SD) allows performing scattering experiments with
Q range between $4.0 \cdot 10^{-5}$ and $2.5 \cdot 10^{-3}$ Å$^{-1}$. A second sample position at 1.3 m SD reaches the Q-range to $1.5 \cdot 10^{-3} - 2 \cdot 10^{-2}$ Å$^{-1}$ and can overlap with the classical pinhole SANS instruments (KWS1). Another “mobile” sample position can be installed to adapt a sophisticated sample environment between 8 and 2 m SD. Thus, by the VSANS at KWS3, the length scale that can be analyzed is beyond 10 μm.$^{94}$

2.2.2 Planning a SANS Experiment

1) Before the SANS experiment (sample preparation and pre-characterization)$^{95}$:

The key to a successful SANS experiment is to know the detailed information of the system studied. As a matter of fact, prior to a SANS experiment, as much information as possible should be collected on the system investigated by complementary methods (light scattering, UV-Vis, TEM, AFM, etc). For example for the solution samples, one may need to know the concentration, scattering contrast, the size range of the scattering, the sample volume, the solvents and buffer before.

2) During the experiment:

Depending on the scatterers size studied, the sample volume available, the temperature and pressure required, the instrument needs to be set up specifically.

○○ Find the optimum Q range$^{95}$: Collimator-sample and detector-sample distances need to be optimized against the size (i.e. presumed radius of gyration) of the scatterers under investigation. If large objects are studied, these distances will generally be chosen large, if smaller objects and higher Q ranges are required, these distances will be chosen shorter.

○○ Instrument calibration: Before measuring samples, the SANS instrument needs to be calibrated at a given detector-collimator setup: centering of the incoming neutron beam, determination of the detector efficiency, evaluation of the background noise and determination of the signal from the sample holder device. These calibrations are done with standard samples specific to neutrons.

○○ Sample measurements: After setting the optimum instrument configuration (Collimator-sample and detector-sample distances, irradiation time, sample temperature, magnetic field etc.), samples are measured on the sample hold with specific sample en-
vironment. In addition, the empty beam (EB) and plexiglass plate as standard are measured for absolute calibration.

3) After the experiment:

The raw data from an experiment of an anisotropic sample should represent the scattering of this scatterer. The data are generally corrected for the sample and instrument geometry, detector efficiency, electronic and backgrounds, and averaged isotropically by the software QtiKWS. Raw data reduction is the first step to do before the data analysis, the principle is shown in the following:

The scattering intensity is related to the scattering cross section by equation 2.1 which is presented before:

\[ I_s = I_0 T_s \Delta \Omega_A s d \frac{d\Sigma}{d\Omega} \]  

(2.1)

By using plexiglass as a secondary standard for calibration, the scattering intensity \( I_{\text{plexiglass}} \) and the measured intensity of the sample \( I_s \) could be rewritten in terms of the scattering cross-section:

\[ \frac{I_s}{I_{\text{plexiglass}}} = \frac{I_0}{I_0} \cdot \frac{T_s}{T_{\text{plexiglass}}} \cdot \frac{\Delta \Omega_s}{\Delta \Omega_{\text{plexiglass}}} \cdot \frac{A_s}{A_{\text{plexiglass}}} \cdot \frac{d_s}{d_{\text{plexiglass}}} \cdot \frac{\frac{d\Sigma}{d\Omega}(Q)_s}{\frac{d\Sigma}{d\Omega}(Q)_{\text{plexiglass}}} \]  

(2.6)

The definition of solid angle is \( \Delta \Omega \approx 1/L^2 \) with the sample-detector distance \( L \). The macroscopic scattering cross section of the plexiglass measurement is \( Q \) independent. Thus, the scattering cross-section of the sample follows the next equation

\[ \frac{d\Sigma}{d\Omega}(Q)_s = \frac{T_{\text{plexiglass}}}{T_s} \cdot \frac{d_{\text{plexiglass}}}{d_s} \cdot \frac{L_s^2}{L_{\text{plexiglass}}^2} \cdot \frac{I_s}{I_{\text{plexiglass}}} \cdot \frac{\frac{d\Sigma}{d\Omega}}{\frac{d\Sigma}{d\Omega}_{\text{plexiglass}}} \]  

(2.7)

Taking into account the measurements of the empty cell scattering \( I_{\text{cell}} \), blocked beam scattering \( I_{BG} \), the final normalized scattering cross section becomes

\[ \frac{d\Sigma}{d\Omega}(Q) = \frac{I_s - I_{BG}}{I_{\text{plexiglass}} - I_{BG}} \cdot \frac{T_s}{T_{EB}} \cdot \frac{I_{EB} - I_{BG}}{I_{EB} - I_{BG}} \cdot \frac{d\Sigma}{d\Omega}_{\text{plexiglass}} \]  

(2.8)
Further data treatment includes the dead time effect correction, which was applied for samples that scatter strongly in the high-Q range.

After the raw data reduction, the data treatment, modeling and interpretation are discussed in the later part.

### 2.3 Small Angle X-Ray Scattering

Small-Angle X-ray Scattering (SAXS) is a non-destructive method for investigating nanostructures in the length scale range from 1nm to several 100 nm concerning shape, size, internal structure, interaction etc. SAXS experiments are representative of an entire sample in mm scale, so SAXS ideally complements methods that provide unique but local information, such as electron microscopy. With sufficiently high and x-ray fluxes, information may be obtained in real time. Thus, it is possible to measure size, shape, and spatial relationship of macromolecules and assemblies as a variety of experimental conditions change (such as temperature, pH, and salinity). Over the last 60 years, the application of SAXS to the structural investigation of materials has made tremendous progress. This progress has been driven by the dramatically improving brightness of synchrotron radiation x-ray sources.

Comparing with the neutron source, the flux of X-rays is usually 200,000 times greater than the same energy of the neutron source. In many times, a laboratory X-ray source can reach the flux as high as a neutron source. Thus, it is very convenient for people to use SAXS as a normal characterization tool instead to go to a synchrotron beamline.

#### 2.3.1 The SAXS instrument

SAXS experiments were mainly carried out by using a laboratory X-ray source instrument GALAXI and HECUS S3-Micro small-angle X-ray scattering instrument.

<table>
<thead>
<tr>
<th></th>
<th>Galaxi</th>
<th>HECUS S3-Micro</th>
</tr>
</thead>
<tbody>
<tr>
<td>Source</td>
<td>Bruker AXS Metaljet source ($\lambda$=1.34 Å)</td>
<td>Cu-Kα ($\lambda$=1.54 Å)</td>
</tr>
</tbody>
</table>
### Small Angle Scattering

<table>
<thead>
<tr>
<th>Photon flux</th>
<th>4 x 10^9 photons/sec</th>
<th>1 x 10^8 photons/sec</th>
</tr>
</thead>
<tbody>
<tr>
<td>Optic system</td>
<td>Pin hole</td>
<td>Point-Focus system</td>
</tr>
<tr>
<td>Beam Size at sample</td>
<td>40 x 40 µm²</td>
<td>70 x 300 µm²</td>
</tr>
<tr>
<td>Detector</td>
<td>Dectris Pilatus 1M with Pixel size of 172*172 µm²</td>
<td>Pilatus 100k detector with Pixel size of 172*172 µm²</td>
</tr>
<tr>
<td>Sample-detector distance</td>
<td>800 mm to 3500 mm</td>
<td>300 mm</td>
</tr>
</tbody>
</table>

Small-angle scattering measurements are conceptually simple as schematically presented in Figure 2.6. The conceptual of the technique is very similar to the SANS instrument mentioned before. Modern synchrotron radiation X-ray beam lines are generally equipped with a tunable fixed exit double monochromator and mirrors for harmonic rejection. The monochromators are usually single crystals with a very narrow bandpass ($\Delta \lambda / \lambda \sim 10^4$), compared to SANS instruments usually with a relatively broad spectral band (FWHM $\sim 10\%$).\(^74, 80\)

![Figure 2.6. Schematic of a typical small-angle x-ray scattering setup.](image)

The operation of the in-house SAXS instrument is much easier than the SANS instrument. They are very similar in principal. Before and after the experiment people need to do similar things to obtain high-quality SAXS data. The main difference for SAXS
sample preparation is to try to make the sample as thin as possible, usually ca. 1 mm or less depending on the sample transmission.

The data reduction is also very similar to SANS data treatment. Conversion of the 2D scattering detector image to the 1D scattering profile usually uses the software Fid2D. Such procedures include detector masking, subtraction of the detector dark current, scaling of each detector pixel for its previously measured sensitivity and the solid angle as seen from the sample, removal of abnormally intense pixels, and integration to yield the 1-D scattering intensity to Q curve.

Water is a good intensity standard for a SAXS experiment. After proper background subtraction, I(Q) is nearly flat scattering over the range 0.1 Å⁻¹ ≤ Q ≤ 0.4 Å⁻¹, with \( \frac{d\Sigma}{d\Omega} \approx 1.64 \times 10^{-2} \text{ cm}^{-1} \) at 25°C. Since SAXS brings higher background from the instrument, an essential step before attempting to understand the normalized data is the accurate subtract of the combined background scattering from the instrument, the sample cell, and the medium or the solvent in the solution samples. In case the SAXS experiments are performed and solution samples are measured in a capillary with a very thin wall, water and solvent are also measured in the same capillary with the same instrument configuration. The final scattering cross section of a sample is:

\[
\frac{d\Sigma}{d\Omega} (Q) = \frac{I(Q)_s - I(Q)_b}{t_s T_s - t_b T_b} \cdot \frac{I_b}{I_w T_w} \cdot \frac{I(\text{EC})}{I(\text{EC})} \cdot 0.0164
\]

Where \( I(Q)_s \), \( I(Q)_b \), \( I(Q)_w \), and \( I(Q)_{\text{EC}} \) are the measured intensity of sample, solution background, water and empty capillary. The bottom term in the high Q range is a flat scattering (Q independent). T are the transmission and the t are the irradiation time.

### 2.4 Analysis of Small-Angle Scattering Data

Apart from a source a Small-Angle-Scattering instrument is conceptually very simple while the SAS data analysis can be quite complex. Unlike an electron micrograph or other direct methods, SAS patterns do not show structure information directly. The result of a SAS experiment is essentially the intensity of the Fourier transform of the contrast and must be interpreted to determine the structure. One cannot recon-
Small Angle Scattering

Struct the exact microstructure uniquely from a SAS pattern because in a scattering experiment only the scattered intensity can be measured and the phase information is lost. Therefore, one cannot be sure that a scattering pattern is due to a particular structure. However, usually something is already known about the system, so that it is often reasonable to assume that if a particular model is shown to fit the scattering pattern, then the model is a correct description of the structure. Nevertheless, many different approaches exist to extract structure information from a SAS pattern.

The following part will show several model-free parameter determinations, model-dependent fitting / model reconstruction and some complex case of SAS analysis on the SAS data.

2.4.1 Model Independent Analysis

The first approach used to understand SAS data consists of a set of general laws and a parameter that yields results right after data reduction. Note that the absolute intensity $I(Q)$ is a shorthand notation for the macroscopic scattering cross section $d\Sigma/d\Omega(Q)$.

**The Scattering Invariant and Porod’s law**

A fundamental relation is that the total small angle scattering from a sample is a constant (i.e. invariant) irrespective of the way the sample density is distributed (Figure 2.7). In the case of a two-phase system, the scattering invariant $Q_I$ is proportional to the volume fractions $\phi_1=\phi$ and $\phi_2=1-\phi$ of phase 1 and 2 and the contrast:

$$Q_I = \frac{d\Sigma}{d\Omega}(Q)dQ = (2\pi)^3 \phi_1 (1-\phi_1)(\rho_2 - \rho_1)^2$$

(2.10)

Thus, equation 2.10 allows for the calculation of the volume fraction of each component in a two-phase system given the contrast, or the contrast given the volume fractions.
**Figure 2.7.** Two systems A and B where the contrast and volume fraction are the same, but the distribution of matter is different. Both are 10% blue and 90% white. On the right is a representation of small-angle scattering curves on system A and B in Log-Log scale of $d\Sigma/d\Omega(Q)$ vs $Q$.

The Porod law describes that the slope of the plot ($\ln [d\Sigma/d\Omega(Q)]$ vs $\ln Q$) represents the interface and fractal dimension of the scattering objects.\(^{69,70,76}\) The Porod region corresponds to a probed range smaller than the scattering objects so that the scattering is probing the local structure. At the Porod region, one can approximate:

$$\frac{d\Sigma}{d\Omega}(Q) \propto Q^{-n}$$  \hspace{1cm} (2.11)

A Porod slope $n = 4$ is obtained for scattering from particles with smooth surface (sharp boundaries, as shown in figure 2.7) and thus the scattering at large $Q$ values solely depends on the total interface area\(^{69,70,72}\):

$$\lim_{Q \to \infty} \frac{d\Sigma}{d\Omega}(Q) = \frac{2\pi(\Delta\rho)^2}{Q^4} \cdot \frac{S}{V}$$  \hspace{1cm} (2.12)

where $S/V$ is the specific surface of the sample. If we consider the systems shown in Figure 2.7 we can see that the specific surface of the left-hand sample will be larger than that of the right-hand one, but they have the same scattering invariant. Thus, equation 2.12 allows for the calculation of the surface of the sample.

Moreover, a Porod slope of $n = 1$ represents a rigid rod; a Porod slope $n = 2$ is obtained for scattering from the thin disk-like particles; whereas a slope $n$ between 3 and 4 characterizes rough interfaces of fractal dimension $D$ with $n = 6-D$. This is called a surface fractal.\(^{69-73,75,76,79,80}\)

**Table 2.3.** An assortment of Porod law behaviors for different shape objects. The pictures are edited and represented from B. Hammouda\(^{79}\) and his lecture note. The red circle is the scattering probed regime.
Furthermore, in the case of polymer coils, the Porod slope $n$ is related to the excluded volume parameter $\nu$ as its inverse $n = 1/\nu$. A slope $n = 2$ is a signature of Gaussian chains in a dilute environment, a slope $n = 5/3$ is for fully swollen coils and a slope $n = 3$ is for collapsed polymer coils. A slope between 2 and 3 is for “mass fractals” such as branched systems (gels) or networks.  

Table 2.2 lists cases of the sample with their Porod slopes. 

Although those model free slope determination of the shape and interface structure are less accurate, they are
very simple and helpful for the first time estimation of the structure of a complex sample.

**Guinier Analysis**

Where the Porod approximation considers the high-\(Q\) limit of scattering, the low-\(Q\) limit can be described using an approximation due to Guinier. The Guinier approximation is formulated as\(^{69-72, 75, 76}\)

\[
\frac{d\Sigma}{d\Omega}(Q) \propto \frac{d\Sigma}{d\Omega}(0)e^{-\frac{(QR_g)^2}{3}}
\]

or

\[
\ln\left(\frac{d\Sigma}{d\Omega}(Q)\right) = \ln\left(\frac{d\Sigma}{d\Omega}(0)\right) - \frac{R_g^2}{3}Q^2
\]

where the radius of gyration, \(R_g\), is the root-mean-square of the distance of all scatterers from the center of gravity. For example\(^{69, 70}\), the radius of gyration of a sphere is given by

\[
R_g = \sqrt[3]{\frac{3}{5}}R
\]

Then there is \(R_{\text{sphere}} \approx 2.58R_g\).

For an ellipsoid of half axes \(a\), \(b\), and \(c\)

\[
R_g = \sqrt[5]{\frac{1}{5}(a^2+b^2+c^2)}
\]

For a cylinder with length of \(L\) and circular cross section of radius \(R\),

\[
R_g^2 = \frac{L^2}{12} + \frac{R^2}{2}
\]

and the equations for other bodies are given in Appendix A1.

So the plot of \(\ln(d\Sigma/d\Omega(Q))\) against \(Q^2\) will have a slope \(-(R_g)^2/3\). Thus, the Guinier plot is widely used to determine the radius of gyration, \(R_g\), in limiting values of \(Q\) range \(QR_g < 1\). Figure 2.8 presents an example for the protein apoferritin in dilute solution in the Guinier plot, from the slope, the precise \(R_g\) of apoferritin is determined as 59.6 Å. By
the way, the intercept \(\ln(d\Sigma/d\Omega(Q = 0))\) can be used to calculate the \(d\Sigma/d\Omega(Q = 0)\) which is called the zero-angle scattering or follow scattering.

![Graph](image)

**Figure 2.8.** Guinier plot for apoferritin dilute solution (2 mg/ml).

**Kratky Analysis**

A Kratky plot \((d\Sigma/d\Omega(Q) \cdot Q^2\) versus \(Q\)) can be used to identify globularity and flexibility of the sample.\(^{69, 71, 76, 79, 80}\) In the case of well-folded globular particles, the Kratky plot will exhibit a “bell-shape” peak at low \(Q\) with a defined maximum and converges to the \(Q\) axis at high \(Q\). While for an unfolded chain like object (Gaussian chains, polymer chains in dilute solution) showing pronounced flexibility, the Kratky plot presents a plateau over a specific range of \(Q\), which do not converge to the \(Q\) axis. The Kratky plot is typically used to analyze the conformation of proteins, but can be used to analyze the random walk model of polymers. Thus, in the chain-like sample systems, Gaussian chains tend to the Kratky plot limit of 1. Stiff chains (rigid rods) increase linearly at high \(Q\) and branched systems (with mass fractal structure) reach a maximum then decrease as \(Q^{-1}\) at high \(Q\).

An example of a Kratky plot is shown for SAS data taken from particles composed of flexible polymers (Figure 2.9).\(^{102}\) For particle-polymer composites, the ability to derive the structural values from SAS data becomes increasingly difficult depending on the
degree to which the particle behaves as a random coil. If the particle is composed of a flexible polymeric extension with a single well-folded domain (partially folded), the scattering on this system can be observed in a Kratky plot where the curve will be described by an initial “bell-shape” peak at low $Q$ followed by an elevated baseline at high $Q$. In many cases, the presence of a plateau will be followed by a slow descent to baseline for partially flexible particles. Further elevation of the high $Q$ baseline towards a hyperbolic-like curve will be observed as a Gaussian chain in solution. The visual features in a Kratky plot provide a means to assess qualitatively the degree of flexibility within the scattering particle.

**Figure 2.9.** Kratky plot ($d\Sigma/d\Omega(Q)\cdot Q^2$ versus $Q$) of scattering data illustrating changes in the behavior of the curve for folded (sphere), partially folded (sphere-random coil) and completely unfolded particles (random coil). For a folded particle, the integrated area under the curve determines the Porod invariant, $Q$, and is scaled by concentration, $c$. The data are adapted from Robert P. Rambo et al\textsuperscript{102}.

The macroscopic scattering cross section for a two-phase system can be divided into a contrast factor (equation 2.5), which describes the difference in scattering length density between the phases, and an integral term, which describes the spatial distribution of the scatterers in the material.\textsuperscript{70, 75, 81, 82} It is possible to describe the distribution of material in terms of a form factor, $P(Q)$, that represents the interference of photons/neutrons scattered from different positions of the same object, and a structure factor, $S(Q)$, that represents the interference of photons/neutrons scattered from different objects. Then the measured intensity (corrected for background and put on an absolute scale) can be expressed as
\[
\frac{d\Sigma}{d\Omega} (Q) = \frac{N}{V} (\Delta \rho)^2 V_p^2 P(Q) S(Q)
\]  
(2.18)

Where \( N \) is the number density of the particles, \( V \) is the sample irradiation volume, \( V_p \) is the volume of the scatterer. The form factor \( P(Q) \) is the square of the complex wave amplitude \( F(Q) \).

\[
P(Q) = \left| F(Q) \right|^2
\]  
(2.19)

Equation 2.19 is the foundation of SAS and all other scattering and diffraction techniques.\(^{81}\) It states that the measured scattering intensity is related via Fourier transformation to the spatial distribution of the nuclei/electrons in the volume of the investigated sample.\(^{69}\) The form factor describes the size and shape of the scatterers and analytical expressions have been derived for many common shapes such as spheres, thin disk, and cylinders. More complex objects can usually be deduced or constructed from these.

In a none interacting system (e.g. particles in a dilute solution), there is structure factor \( S(q) = 1 \). In a solution, the structure factor is given by\(^{72,75}\)

\[
S(Q) = 1 + 4\pi N \int_0^\infty [g(r) - 1] \frac{\sin(Qr)}{Qr} r^2 dr
\]  
(2.20)

where \( g(r) \) is the pair correlation function for the scattering objects and \( \ln(g(r)) \) is directly related to the potential energy function that describes the inter-particle interaction.\(^{69,72,75,83}\) The structure factors can either be obtained by a model fitting or a designed scattering experiment.

**The Form Factor for Uniform Spheres**

For a monodisperse spherical particle with radius \( r \), the form factor is described as below\(^{69}\)

\[
P(Q) = \left[ \frac{3(\sin(Qr) - Qr \cos(Qr))}{(Qr)^3} \right]^2
\]  
(2.21)

when included a contrast, background, and volume, an example of sphere form factor is shown in Figure 2.10. The form factor of the sphere with a radius \( r = 100 \text{ Å} \) shows two distinguished \( Q \) regimes: the \( Q \) range smaller than 0.01 \( \text{Å}^{-1} \) (\( Qr < 1 \)) is the Guinier region where the scattering curve slope close to 0 indicates the size of the spherical
particles, while in the high Q region the scattering with oscillations and the decay follows a power law of $Q^{-4}$ indicating a sharp interface.

![Graph showing the form factor of spheres of radius 100 Å](image)

**Figure 2.10.** Form Factor of spheres of radius 100 Å.

### The Form Factor for Cylinders

For a monodisperse rod particle with radius $r$ and length $L = 2H$ ($L \gg r$), then

$$P(Q) = \int_0^{\pi/2} f^2(Q, \alpha) \sin(\alpha) d\alpha$$

$$f(Q, \alpha) = j_0(QH \cos \alpha) \frac{J_1(Qr \sin \alpha)}{Qr \sin \alpha}$$

$$j_0(x) = \sin(x) / x$$

$$V_{cyl} = \pi r^2 L$$

where $J_1(x)$ is the first order Bessel function. Here $\alpha$ is defined as the angle between the cylinder axis and the scattering vector, $Q$. The integral over averages the form factor over all possible orientations of the cylinder with respect to $Q$.\(^{69,71}\)

When including a contrast, background, and volume, an example of a rod form factor is shown in Figure 2.11. The form factor of the rod with a radius $r = 30$ Å and $L = 500$ Å shows three distinguished Q regimes: in the Q range smaller than 0.01 Å\(^{-1}\) ($QR < 1$) is
the Guinier region where the scattering curve slope close to 0 indicates the $R_g$ of the rod particles; in the intermediate $Q$ region the scattering shows a slope of -1 indicates a typical rod-like particle; in the high $Q$ region with oscillations and the decay follows a power law of $Q^{-4}$ indicating a sharp interface.

**Figure 2.11.** Form Factor of rod of radius 30 Å and length 500 Å.

In the case of $r >> L$, the cylinder form factor becomes to the thin disk form factor. Figure 2.12 presents a thin disk form factor with radius 500 Å and thickness 30 Å. The three distinguished $Q$ regimes are similar to the rod form factor. However, the differ-

**Figure 2.12.** Form Factor of thin disks of radius 500 Å and thickness 30 Å.
ence is that in the intermediate Q regime the slope of the scattering is close to -2 indicating a 2D thin disk like scattering.

### 2.4.2 Some Empirical Expressions

Standard plots (Guinier, porod and Kratky et al.) give the first order interpretation of SAS data. Model fitting gives a more detailed approach at obtaining SAS results. In the real systems, some models are not always available or too complex to use. An intermediate approach consists in using empirical models/expressions that reproduce the main trends observed in the SAS data. Some of these models are involved in the thesis results discussion and are described here.

**The Beaucage Expression**

The Beaucage empirical expressions are able to reasonably approximate the scattering from many different types of particles, including fractal clusters, random coils (Debye equation), ellipsoidal particles, etc. The Beaucage expression is given according to:

\[
\frac{d\Sigma}{d\Omega}(Q) = \frac{d\Sigma}{d\Omega}(0) \exp(-u^2 / 3) + P_a [(\text{erf}(u / \sqrt{6}))^3 / Q]^{\alpha}
\]

representing a combination of Guinier’s and Porod’s laws describing the scattering at low and large Q, respectively. More quantitatively both approximations are valid for the parameter \( u = R_gQ \) smaller or larger than 1, u representing the product of radius of gyration \( R_g \) and scattering vector Q. Guinier’s law has the shape of a Gaussian function whereas for Q larger than \( 1/R_g \) (\( u > 1 \)) a power law according to \( d\Sigma / d\Omega(Q) = P_a Q^{-\alpha} \) is often observed. Those empirical expressions can also be used in a multi-level model for a sample having a multi-scale structure.

An example of Beaucage expression fitting of polymer dense clusters is shown in Figure 2.13. It is difficult to find a precise model to fit such kind of data. Thus from the Beaucage fitting results either the \( R_g = 3 \, \text{Å} \) and a slope of -2 at high Q is easily obtained and can be used to image the shape of the scatterers.
The Correlation Length Model

The following empirical functional model developed by B. Hammouda\textsuperscript{104} is made of two terms. The first term describes Porod scattering from clusters (exponent = n) at small Q and the second term is a Lorentzian function describing scattering from polymer chains (exponent = m) at larger Q. The second term $C/[1+(Q\xi)^m]$ characterizes the polymer/solvent interactions and therefore the thermodynamics. The two multiplicative factors A and C, the incoherent background BKG and the two exponents n and m are used as fitting parameters. Note that when m = 2, this functional form becomes the familiar Lorentzian function.\textsuperscript{79, 104}

\[
\frac{d\Sigma}{d\Omega}(Q) = \frac{A}{Q^n} + \frac{C}{1+(Q\xi)^m} + BKG
\]  

Figure 2.14 presents the correlation length model with a correlation length of 20 Å, Lorentzian exponent of 3 and porod exponent of 3. The correlation length $\xi$ gives a good estimate of the average entanglement length for the polymer in solution. The Porod exponent $n = 3$ points to a “mass fractal” for polymer chain clusters. In this case of the polymer clusters, the first term $A/Q^n$ becomes a key parameter to evaluate the clustering strength.
Small Angle Scattering

Figure 2.14. The correlation length model with a correlation length of 20 Å, Lorentzian exponent of 3 and porod exponent of 3.

2.4.3 A Small-Angle Scattering Analysis of a Hierarchical Structure

In nature, biominerals are unique organo-mineral nanocomposites, organized at several hierarchical levels, from nano- to macroscale. Even some man-made composite materials, often are more complex with organic and inorganic phase structure on different scale levels (see figure 2.15). Small-angle scattering from complex structures often involves understanding the relationship between related structural features observed at different size scales from nano- to macroscale. However, the traditional scattering methods are generally not sufficiently flexible to describe complex hierarchical structures in one simple way and it is difficult to accurately analyse the data.\textsuperscript{105, 106}

Figure 2.15 presents a typical example of small angle scattering study on a complex material with structural information range on five-length scales. Scattering analysis that describes hierarchical structures: (I) large particles with Guinier behavior in the ultra-small Q range, the size of the large structure is in the micrometer scale; (II) Mass fractal due to the branched large-particles-clusters, the Porod slope is around -3; (III) Self-assembled nanoparticle chains with a Porod slope of -1.5; (IV) Scattering from the primary nanoparticles with a sharp interface (high Q Porod slope of -4) (V) Wide angle scattering from atomic structure. Thus on hierarchical materials the scattering is quite complex. Analyzing the structure may cover a large Q range and combine the use of many other methods to support the data analysis. For complex hierarchical systems where a simple discrete structural model is not available, one may consider a separate
analysis of the data in different Q ranges with considering the interference. In some cases, a unified fit combining several Guinier-Power law scattering ranges with $R_g$ transitions by a multi-level Beaucage expression is a success. However, this unified approach often offers the only reasonable approach to understanding small-angle scattering. The unified function offers the opportunity to resolve scattering features obscured by the overlap of structural levels.  

![Small Angle Scattering](image)

**Figure 2.15.** A Small angle scattering (SAS) combined with Ultra-SAS and wide angle scattering investigated on structural information range on five-length scales. Structural features at larger length scales are observed at smaller Q. The picture of the materials is partly adapted from Edler.  

### 2.4.4 Anisotropic Small Angle Scattering

So far we have discussed isotropic samples where the scattering is isotropic at the same Q value. The scattering patterns depend on the shape, size ($P(Q)$, form factor) and ordering ($S(Q)$, structure factor) of the scattering particles resulting in different shaped SAXS scattering, mostly in circles (isotropic scattering) on the 2 D SAXS detector. However, anisotropic SAXS scattering arises if the studied system contains anisotropic inhomogeneities, elongated or flattened, or certain predominant arrangement of molecules. The scattering pattern is the reciprocal “image” (Fourier transform) of the size and the arrangement of the particles (real space). If the particles in the sample are randomly oriented, the 2D scattering pattern of such a system would be a circular aver-
Small Angle Scattering

age and isotropic. While the particles have more or less orientation, the 2D scattering pattern becomes anisotropic. The pattern contains additional information related to the structure which makes the small angle scattering analysis quite complicated.

**Figure 2.16.** A Small angle scattering (SAS) on rod-like micelles without shear (A) and shear-induced alignment along the horizontal direction (B) and perpendicular to the paper direction (C). In the middle are the 2D SAS scattering patterns corresponding to the real system. The bottom curves are the 1D data integrations from the 2D data. The 2D images of sample A and C are treated with radial integration. Pixels with a certain radial distance (Q) from the beam center are depicted by circles. The 2D image of sample B is integrated with a degree (χ, circle) at a fixed Q value.

An example (similar as Edler\textsuperscript{108}) on isotropic and anisotropic scattering is presented in Figure 2.16. Rod-like micelles are randomly oriented with an isotropic 2D scattering pattern (Figure 2.16 A). The 1D scattering is a typical rod form factor. From this data, we will know the size and shape of the particles. While with special force on the system (Figure 2.16 B), in the case of shear on the horizontal direction, the rod-like micelles become aligned along the shearing direction. Thus, the scattering becomes anisotropic and the scattering intensity has a fluctuation along a fixed Q value (I(χ)-χ). In Figure 2.16 C, the shear direction becomes perpendicular to the paper, although the micelles along the shear direction, the scattering is isotropic due to missing orientation in the detector plane. However, the scattering is quite different from the first case because in sample C only the rod cross section of the micelles is seen. Thus, a much smaller struc-
ture is found from the 1D scattering of sample C. The length of the rod has been lost due to the alignment direction along the beam direction.

For a sample including a large number of particles with the order on the nanoscale, a view from the macro scale shows that they are isotropic. Examples are a superlattice of small particles in solution or particles. There is a scattering pattern with a peak in 1D or circle in 2D. This can be analysed by the Bragg law \( d = 2\pi/Q \). However, when the superlattice is in the form of big single crystals or in the form of films with long-range orientation, the scattering becomes isotropic. Then the analysis of the data becomes complicated in the 1D pattern. Usually, the data are studied in the 2D images.

### 2.4.5 Shape Reconstruction from SAS Data

Using ab initio modeling and rigid body modeling it is possible to reconstruct a low-resolution shape model of a particle from the measured data and without any a priori information.\(^{71, 80, 109}\) The approach starts with an initial guess of the shape (such as a sphere, with input of some parameters e.g. \( R_g \), symmetry), represented by a collection of dummy scatterers. Then the guessed shape is repeatedly refined against the measured data until it fits well with the experimental data. After that the most probable shape structure is produced and usually stored as a PDB file with atomic positions. The rigid body modeling approaches utilize the scattering from the domains/subunits.\(^{110, 111}\)

![Figure 2.17. SAS and 3D model reconstruction from for DNA cages. (a) SAXS experimental data (hollow circle) and model fit (solid lines). (b) The octahedral model is obtained from the SAXS data. (c) Overlay of SAXS model and cryo-TEM reconstruction in three different views.\(^{112}\)](image-url)
An example on DNA cages investigated by SAXS and the 3D model reconstruction model is shown in Figure 2.17. The analysis of the SAXS data shows that the sample contains a very well-defined hollow cage. The low-resolution modeling approach using spheres for representing the C2* sequence of the DNA structure gave a good fit to the data (Figure 2.17 a) resulting in the model shown in Figure 2.17 b. The model reveals that the distance from the center of the cage to the center of the double-stranded DNA is \(62.5 \pm 0.7 \text{ Å}\) and the maximum diameter of the model is \(\sim 155 \text{ Å}\), which is in agreement with the expected size. The inner diameter obtained from the SAXS is \(\sim 100 \text{ Å}\) and the side of the apertures is around 60 Å in diameter.

### 2.5 Summary

In the above discussion, the basic theory, instrument parameters, SAS experiments, SAS data reduction and further data analysis by model dependent/independent methods were well described. However, the studied system is quite complicated: The structure includes both organic and inorganic phases that interact with each other; The sample hierarchical structures are covering a large length scale from 1 Å to 10 µm; The mineralization mechanism is quite complicated that the two component structure may change during the mineralization process; The related materials are sensitive to the environment. In order to well study the system concerning in the thesis, here I am using a schematic diagram (Figure 2.18) to illustrate the organization of the SAS investigation of the work.
Figure 2.18. Schematic diagram illustrating the organization of SAS investigations on the magnetite mineralization in gelatin hydrogels.
3 Gelatin Hydrogel Matrices

3.1 Introduction

Gelatin is a water soluble polypeptide derived from insoluble collagen through hydrolysis. The fibrous collagen molecule is formed by three individual polypeptide strands and has the conformation of a triple-helix. The collagen (collagen type 1) extracted from skin and bones is composed of α-chains with a molecular mass ~95 kDa, width ~1.5 nm and length ~300 nm. Hydrolysis separates the collagen triple-helix into three polypeptide strands. These are further hydrolyzed to gelatin. Each polypeptide strand with left-handed proline helix conformation contains between 50-1000 amino acids (Figure 3.1). An aqueous solution of ~0.5-50 wt% gelatin at ~40°C is in a sol state and forms when cooled to lower temperatures a thermo-reversible gel due to the recovery of collagen-like right-handed triple helices via a transition from a random coil to triple-helix conformation. These helical segments pack and form the crosslinks. Higher levels of these triple helices result in stronger gels due to higher numbers of packed gelatin helices. Dry gelatin films containing greater amount of triple helices content swell less in water and are consequentially much stronger.

Figure 3.1. Schematic illustrating the structure of gelatin. (A) A typical Molecular structure unit of gelatin. (B) The molecular structure of collagen in a space filling and (C) ball-stick model. The structure is obtained from a reconstruction of a PDB file (1CLG) which is from Chen.
There are two types of gelatin. Type A, with the isoelectric point (IEP) of 8 to 9, is derived from collagen with acid pretreatment. Type B, with IEP of 4.8 to 5.2, is the result of an alkaline pretreatment. Under the alkaline pretreatment, asparagine and glutamine residues are converted to their respective acids resulting in higher viscosity with an isoelectric point of 4.8 to 5.2. When the pH is close to the IEP, gelatin is neutral in charge. If the pH is higher than the IEP, the gelatin is negatively charged while lower pH gelatin becomes positively charged.

Generally, gelatin hydrogels are attractive soft materials for biological applications owing to their hydrophilic insoluble properties with various functional groups (-NH$_2$, -COOH) and biocompatibility. Cavities inside the hydrogel could act as a protein scaffold, which, therefore, is an ideal natural protein assembly to control the spatial and structural order of the mineral. The last property makes the gelatin hydrogels a convenient platform to model a material via magnetite mineralization in order to achieve properties of Chiton's tooth.

Small-angle neutron scattering (SANS) is an important non-destructive technique for the determination of structural length scales from 1 nm to several 100 nm. SANS can probe the internal structure of materials in situ, such as samples in solution, at elevated temperatures, under pressure. Thus, SANS is an outstanding tool for quantitative evaluation of hydrogel networks. However, there are few SANS experiments dealing with biopolymer hydrogels, e.g. gelatin hydrogels. The structure is more complex due to the multi-scale (hierarchical structure from nanometer to micrometer), thermal and environment sensitivity.

In this part, we explored the structure of gelatin hydrogels ranging over a large length scale by using small (SANS) and very-small (VSANS) angle neutron scattering in conjunction with small (SAXS) and wide (WAXS) angle X-ray scattering as well as rheology/thermal measurements. We aimed to investigate the gelatin structure in-situ and probe the structure changes upon a concentration, temperature, pH, salts change which happens during the magnetite mineralization process. This structure characterization may help for deep understanding of the gelatin role on the mechanism of the bio-inspired mineralization.

The following presented data were performed in a collaboration with different groups in a priority program project of the DFG (SPP 1569 Generation of multifunctional inor-
ganic materials by molecular bionics). Samples synthesis and parts of non-scattering characterization are closely collaborated with Maria Siglreitmeier (a Ph.D. student in AG Cölfen, Uni Konstanz). Part of the presented results in this Chapter were adapted from the following publications:


### 3.2 Experimental Section

#### 3.2.1 Materials

Materials: The following commercially available chemicals were purchased and applied in the synthesis without further purification: FeCl$_2$·4H$_2$O (Sigma-Aldrich), FeCl$_3$·6H$_2$O (Sigma-Aldrich), HCl (37%) (Sigma-Aldrich), DCI (35 wt. % in D$_2$O, 99 atom % D, Sigma-Aldrich), NaOH (Sigma-Aldrich), NaOD (40% in D$_2$O, Sigma-Aldrich), CaCl$_2$ (Sigma-Aldrich), CuCl$_2$ (Sigma-Aldrich), CoCl$_2$ (Sigma-Aldrich), NaCl (Merk), D$_2$O (99.8% D) (ARMAR Chemicals), Gelatin Type B (~225 Bloom, Sigma-Aldrich), 4-chloro-m-cresol (Fluka), Methanol (VWR), Sodium acetate buffer (0.02 M, pH 5.0).
the preparation of the reactant solutions double-distilled and deionized (Milli-Q) water was used. All iron including solutions were degassed with argon before use.

### 3.2.2 Synthesis of Gelatin Hydrogels

Different amounts of gelatin were allowed to swell in water for 24 hours at $5 \pm 1^\circ$C. Homogeneous solutions were prepared by heating the gels for 2 hours at $45^\circ$C. In each case, 10 mL of solution was filled into a glass dish ($D = 11$ mm) and allowed to form a hydrogel film with a thickness $\sim 1$ mm. To avoid decomposition by bacteria, a 5 wt% solution of 4-chloro-m-chresol in methanol was added (0.15 mL per 1 g of dry gelatin). All the samples for SANS-VSANS measurements were prepared in $D_2O$ or mixture of $D_2O$ and $H_2O$. The metal ions were introduced into gelatin hydrogels by either directly adding in the gel synthesis process (Na$^+$, Ca$^{2+}$) or soaking the hydrogels in ion (0.3 M Fe$^{3+}$) containing solutions at $5 \pm 1^\circ$C.

### 3.2.3 SANS and VSANS Experiments

SANS and VSANS experiments were carried out at, respectively, the KWS-1$^{91}$ and KWS-3$^{94}$ diffractometers operated by Jülich Centre for Neutron Science (JCNS) at Heinz Maier-Leibnitz Zentrum (MLZ) in Garching, Germany. Four configurations were used at KWS-1, namely the sample-to-detector (SD) distances of 1.3, 2, 8 and 20 m, the collimation length of 8 and 20 m, and wavelength of 7 Å ($\Delta \lambda/\lambda = 10\%$). These settings allowed covering a Q-range from 0.002 to 0.35 Å$^{-1}$. A two-dimensional position sensitive detector was used to detect neutrons scattered from samples. In order to cover the broader length scale of the network structure, very-small angle neutron scattering (VSANS) experiments were carried out at the KWS-3 diffractometer using the parabolic mirror as an optical element, and covering the smaller Q range from 0.0001 to 0.0025 Å$^{-1}$. Hydrogels were filled in rectangular quartz cells with path-length of 1 or 2 mm. Plexiglas was used as a secondary standard to calibrate the scattering intensity in absolute units at KWS-1 and direct beam method was used at KWS-3. The data correction and calibration were performed using the software QtiKWS$^{96}$. Fitting the SANS/VSANS data was done using software modules provided by JCNS QtiKWS and NIST Igor$^{127}$ analysis packages.
3.2.4 SAXS Experiments

SAXS experiments were carried out with a HECUS S3-Micro and GALAXI\textsuperscript{97} small-angle X-ray scattering instrument. The HECUS S3-Micro instrument uses Cu K\textsubscript{α} radiation (0.154 nm) produced in a sealed tube. The GALAXI instrument uses a Metaljet source ($\lambda=1.34$ Å). Gel samples were placed in Hilgenberg quartz capillaries with an outside diameter of 1 mm and wall thickness of 0.01 mm. The scattered intensity was corrected with the transmission of the samples calculated considering the absorption of the sample and that of the capillary. The dry gel samples were cut to a thin film with a thickness 1 mm and measured directly. The scattered X-rays were detected with a two-dimensional multi-wire area detector and afterwards converted to one-dimensional scattering by radial averaging and represented as a function of momentum transfer vector $Q$ similar to the SANS experiments.

3.2.5 Thermal Analysis

**Differential scanning calorimetry (DSC):** Unfolding, glass transition, solid-melting of gelatin samples with different concentration were measured by differential scanning calorimetry (DSC 8500, Perkin Elmer).

**Thermogravimetric analysis (TGA):** The H\textsubscript{2}O content of the dry gelatin was determined by means of TGA (Netzsch, Selb, Germany). Measurements were carried out at a heating rate of 5 K/min under a constant N\textsubscript{2} flow. Samples were scanned from 293 K to 473 K.

3.2.6 Rheological Experiment

Rheological properties of gelatin hydrogels (18 wt% in D\textsubscript{2}O) were measured at shear deformation by Physica MCR501 (Anton Paar, Graz, Austria) rheometer using the couette (concentric cylinder) working unit (Titanium Cup Diameter is 49 mm and thickness is 1 mm).

3.2.7 Atomic Force Microscopy (AFM)

Non-contact mode atomic force microscopy (AFM) using an Agilent 5500 microscope was employed to study the 3D structure of the gelatin dry films.
3.2.8 Powder X-ray Diffraction (XRD):

XRD patterns were recorded on an image plate camera (Model G670, Huber, Cu Kα1 radiation, λ = 1.54 Å, quartz monochromator, Guinier camera). Diffraction data were collected between 3° and 100°.

3.3 Results and Discussion

3.3.1 General Macroscopic Properties of Gelatin Hydrogels

The gelatin Type B used in the experiments has a molar mass of approximately 25-50 kDa. The thermal treatment (T ~45°C) occurs in the presence of water (includes D2O or mixtures of H2O/D2O) and is necessary in order to reduce both hydrogen and electrostatic interactions.

![Figure 3.2](image)

**Figure 3.2.** The viscosity versus temperature results of gelatin hydrogels (18 wt% in H2O). In the inset, \( T_g \) is the so-gel transition temperature. The viscosity is measured by an oscillating rheometer with a fixed shear at 250 s\(^{-1}\).

When the temperature is cooled below roughly 35°C, the gelatin chains undergo a progressive conformational change of coil-to-helix transition\(^{116}\). As a result, a thermo-reversible gel\(^{128}\) is formed accompanied by a progressively increasing solution viscosity (Figure 3.2). Such a sol-gel transition occurs due to the formation of polypeptide inter-
molecular hydrogen bonds between carbonyl oxygen and amide hydrogen (crosslink). At the same time, gelatin loses its solubility at cooling owing to the coil-to-helix transition.\textsuperscript{129} From the inset of Figure 3.2 of the rheology viscosity as a function of temperature plots, there is a critical point at 30°C which is close to the sol-gel transition temperature $T_g$.

![Figure 3.3](image)

**Figure 3.3.** The loss modulus $G''$ and the storage modulus $G'$ versus temperature of gelatin hydrogels (18 wt% in H$_2$O). $T_g$ is the sol-gel transition temperature. The $G''$ and $G'$ are measured by an oscillating rheometer with a fixed shear rate at 47 s$^{-1}$.

The viscous and elastic properties in rheology measurements are represented by the so-called viscosity or loss modulus $G''$ and the elastic properties by the storage modulus $G'$. During a typical gelation process for a physical gel, there is a geometric point where the loss modulus $G''$ and the storage modulus $G'$ are in equilibrium.\textsuperscript{119} This crossover point of $G'$ and $G''$ on the cooling curve is defined as the sol-gel transition temperature (gelation point) $T_g$.\textsuperscript{130} Figure 3.3 shows the storage modulus $G'$ and the loss modulus $G''$ as a function of cooling temperature with a fixed shear rate. Both of the $G'$ and $G''$ increased with decrease of the temperature and show crossover at around 29.7°C. This crossover point indicates a gelation point which is quite close to the value obtained from figure 3.2. The increasing of $G'$ upon cooling the system implies that at low temperature the hydrogels become more rigid than that high temperature, i.e., become more “solid like”.

- 51 -
Figure 3.4. The heat flux versus temperature DSC curves of (A) gelatin hydrogels (in H$_2$O) as a function of hydrogel concentration (by weight) and (B) gelatin 18 wt% in D$_2$O and H$_2$O. $T_m$ is thermal transition midpoint.

The choice of working temperature with gelatin hydrogels is extremely important due to the thermal sensitive properties of gelatin hydrogels. Thus, characterization of the thermal transition temperature especially the melting temperature is important for optimally using gelatin as a mineralization medium. Experimental results of the use of differential scanning calorimetry (DSC) (Figure 3.4 A) indicate that the transition temperature $T_m$ between 27 and 31°C is a function of gelatin concentration. The peak positions in the
DSC curve is the gelatin thermal transition midpoint $T_m$, which is slight higher than the gelation point, i.e. the $T_g = 29.7^\circ C$ (from rheological measurement) while $T_m = 30^\circ C$ (DSC results) for gelatin 18 wt% in $H_2O$. Since many of the gelatin samples will be measured in $D_2O$, we checked the thermal properties of gelatin hydrogels by using $D_2O$ as the solvent shown in Figure 3.4 B. The DSC results indicates that gelatin in $D_2O$ and $H_2O$ has similar thermal properties and show the same thermal transition midpoint at $T_m = 30^\circ C$.

![Figure 3.5. The phase diagram of gelatin (in $H_2O$) as a function of concentration and temperature. $T_m$ is the DSC thermal transition midpoint. $T_{gel}$ is defined by the temperature at that the sol becomes significantly viscous and loses the flowability. $T_{sol}$ is the temperature at that the gel becomes soft with flowability. The data points of the phase diagram were obtained by DSC, rheology, and microscopy measurements.](image)

From above rheology and thermal analysis, Figure 3.5 presents the phase diagram of gelatin (in $H_2O$) as a function of concentration and temperature (by heating the hydrogels). For gelatin concentration below 3 wt% rheology and thermal analysis do not show with confidence the transition temperature. Thus, the results for concentrations of 1 wt% and 0.5 wt% are obtained by a microscope observation.

The temperature-concentration phase diagram shown in Figure 3.5 has four distinct regions: gel, sol, glassy phases, and sol-gel transition region. The gelation occurs above
0°C for all concentrations above a critical concentration of 0.5 wt% gelatin which was widely reported as the critical overlap concentration\textsuperscript{131}. Below the critical point there is only a sol phase. Above the critical concentration, the system shows the sol-gel temperature-induced transition with some coexisting temperature region. Further increasing the gelatin concentration above 50 wt%, the phase becomes much more dense thus a glassy phase appears\textsuperscript{132}. Within the sol-gel region between 0.5 wt% and 50 wt% of gelatin contain, the sol-gel transition curve shows the upturn of the convex-shape, implying the existence of an upper critical solution temperature in the gelatin solution below gelation temperature.\textsuperscript{133} Increasing the gelatin concentration below 6 wt% significantly increases the temperature of the sol-gel transition, while above 6 wt%, the transition temperature increases slowly. Thus, the higher the gelatin concentration, the higher transition temperature, and the broader the sol-gel transition region appears in the phase diagram.

As an organic matrix, the hydrogel strength is crucially important. For gelatin hydrogels, the helix amount will influence the gel strength a lot. Thus, we found and selected optimal conditions for SAS structural investigations of 6 wt% \( \leq c_{\text{gelatin}} \leq 18 \) wt% at a temperature of \( \sim 20^\circ \text{C} \).

### 3.3.2 The Micro Structure of Gelatin Hydrogels

The structure of the hydrogels of various gelatin concentrations between 0.1wt% and 30 wt% in D\textsubscript{2}O was determined at the SANS and VSANS diffractometers at room temperature within the gel-phase of the phase diagram. The scattering cross-section of the investigated samples was obtained within the scattering vector from very small \( Q \) of the order of \( 10^{-4} \) up to 0.3 Å\(^{-1}\), covering length scales from 20 Å up to 6 µm.
Figure 3.6. SANS/VSANS scattering profile of 18w% gelatin in D$_2$O measured at T = 20 ±2°C. The light-blue solid line (Q < 0.01 Å$^{-1}$) represents fitting by equation 3.1 of the Beaucage expression. The dark-blue solid line (Q > 0.01 Å$^{-1}$) represents fitting by equation 3.3 of the fractal cylinder model. The dash-dot-red line represents a cylinder form factor with polydispersity of 0.2. The left inset picture is an AFM phase image showing gelatin large clusters (dried gelatin sample). The right bottom inset picture is a cylinder model for the gelatin triple helix.

In Figure 3.6 the scattering cross-section of 18wt% gelatin in D$_2$O is presented as obtained at SANS and VSANS instruments. The scattering can be divided into two regimes. Regime I (Q < $Q_c$ ≈ 0.025 Å$^{-1}$, where $Q_c$ is the knee point) describes scattering from large gelatin clusters, whereas Regime II (Q > $Q_c$) reveals information from the internal gelatin structure. The scattering below 0.01 Å$^{-1}$ in regime I (Q < $Q_c$ ≈ 0.024 Å$^{-1}$) shows for the sample the Guinier regime of scattering of a large-scale inhomogeneous structure. The low Q scattering can be well described by the solid line representing the best fit of the data using the Beaucage expression$^{103}$ which is given according to:

$$\frac{d\Sigma}{d\Omega}(Q) = \frac{d\Sigma}{d\Omega}(0) \exp(-u^2/3) + P_a[(\text{erf}(u/\sqrt{6}))^3/Q]^{\alpha}$$

(3.1)

representing a combination of Guinier’s and Porod’s laws describing the scattering at low and large Q, respectively. The dark blue line in Figure 3.6 corresponds to the fit of
the scattering cross-section of the 18 wt% gelatin sample in “Regime I” by Eq.3.1: radius of gyration of \( R_g = 1590 \pm 30 \) Å and mass-fractal dimension \( \alpha \approx 2.7 \) were obtained. By assuming of the spherical form of the large clusters, an average diameter of them above 4100 Å is calculated. The data is in rather good agreement with the AFM result which presents elongated particles with length of \( \sim 0.4 \) μm and diameter of \( \sim 0.15 \) μm (inset of figure 3.6). Forward scattering of the Guinier’s law \( d\Sigma/d\Omega(0) \), amplitude of the Porod’s law \( P_\alpha \), as well as other above mentioned parameters of this fit are listed in Table 3.1.

In order to visualize the large clusters, in Figure 3.7 (A) a 3D representation of the structure was reconstructed using the rapid \textit{ab initio} shape determination program DAMMIF\textsuperscript{134} from EMBL Hamburg. The reconstruction was performed inside the search volume of maximum dimension \( D_{\text{max}} \) calculated from the pair distance distribution function \( p(r) \) determined for the measured SANS data by the GNOM program. The reconstructed model was obtained without setting any restrictions on the symmetry and anisometry of the particles.\textsuperscript{135} In figure 3.7 (A), the 3D model is placed in a cubic cell with a side length of 3000 Å indicating that the cluster has a shape of an approximate ellipsoid with a longest diameter \( \sim 3450 \) Å and the shortest diameter \( \sim 2000 \) Å. The model also shows that the cluster has a dense inner core and a loose surface shell. This 3D structure is in reasonably good agreement with the fitting results that the particle like cluster has a fractal surface.

The scattering above 0.01 Å\(^{-1}\) including regime II (\( Q > Q_c \approx 0.024 \) Å\(^{-1}\)) and part of regime I follows a two power law scattering crossing over from \( Q^{-1} \) (higher \( Q \) part) and \( Q^{-3} \) (lower \( Q \) part in the regime II) as \( Q \) decreases. The crossover at \( Q_c \) tracks a change from a regime in which scattering from single rods dominates (at high \( Q \)) to one in which collective effects dominate (at low \( Q \)). Thereby for gelatin hydrogels, the scattering indicates a rod-like structure of gelatin helix bundles with fractal-like aggregation.

At the cross over \( Q_c \), \( R_m = 2\pi/Q_c \) is considered as a length scale between the large clusters and the single rods which could be the 3D-cage size of the hydrogel network.
Figure 3.7. Schematic representation of gelatin structure from larger scale to molecular level: (A) Gelatin large scale clusters constructed from the scattering data in regime I by a rapid ab initio shape determination program DAMMIF. (B) Sketch of gelatin hydrogel structure with large clusters and gel network. (C) Cage like Gel matrix. (D) Gelatin triple-helix and random coil. (E) & (F) Macromolecular structure, a collagen-like triple-helical structure reconstructed from Chen et al. (Protein Database code 1CLG)

The scattering above 0.01 Å⁻¹ can also be fitted by the Beaucage equation producing the \( R_g \) and high Q power law slope \( \alpha \). However, in the case of gelatin 18 wt%, most of the gel network is constructed by rigid rod-like triple-helices. The high Q scattering (Q > 0.01 Å⁻¹) can be well described by the dark-blue solid lines representing the best fit of the fractal-cylinder model. The model is a combination of a cylinder form factor \( F(Q)_{\text{cyl}} \) (equation 2.22, 2.23) and a fractal structure factor. The structure factor, \( S(Q)_{\text{fractal}} \), for a fractal rod-like network has previously been derived

\[
S(Q, D, \Xi, r0) = 1 + \frac{D \exp(\Gamma(D-1)) \sin(D-1) \tan^{-1}(\Xi)}{(Qr_0)^D [1+(\Xi)^{-2}]^{(D-1)/2}}
\]  

(3.2)
where \( \Gamma(x) \) is the gamma function, \( r_0 \) is the gauge of measurement and \( \Xi \) is the characteristic length of the fractal object above which the weighted-average inter-distance of the object can no longer be described as fractal. The mass fractal dimension \( D_f \) is the negative value of the power law exponent \( n \). Then combining the form factor and the structure factor with the contrast, volume fraction \( (\phi) \), volume of the particles \( (V_p) \) and background \( (\text{BKG}) \) there is

\[
\frac{d\Sigma}{d\Omega}(Q) = \phi V_p (\Delta \rho)^2 P(Q)_{\text{cyl}} S(Q)_{\text{fractal}} + \text{BKG} \tag{3.3}
\]

The obtained values of the fitted parameters from equation 3.3 are given in Table 3.1. Inspection of the returned values of fitting results in Table 2 clearly indicates that long rod-like objects with a detected length of \(~190 \text{ Å}\) and cross section diameter of \(~9.8 \text{ Å}\) \((D=2r)\) aggregate in a mass fractal network. Such a value on rod diameter is fairly in agreement with the collagen helix derived from crystallographic data as presented in Figure 3.7 (E) and (F). The characteristic length of \(~249 \text{ Å}\) is very close to the value calculated from the crossover \( Q_c \) might be the size of the hydrogel 3D cage which is constructed by the gelatin helices. Thus the three length parameters, Characteristic length \( \Xi \), 3D cage size \( R_m \) and the length of gelatin helices \( L \) are correlated with the crossover point \( Q_c \).

| Table 3.1. Parameters of Gelatin hydrogels in D\(_2\)O obtained from the SANS/VSANS data. |
|---------------------------------|----------------|----------------|----------------|
| **Low Q parameters** | **Numerical values** | **High Q parameters** | **Numerical values** |
| \( d\Sigma/d\Omega(0) \ / \text{cm}^{-1} \) | 2500 \( \pm \)50 | Fractal dimension \( / D_f \) | 2.9 \( \pm \)0.1 |
| \( R_g \ / \text{Å} \) | 1590 \( \pm \)30 | Characteristic length, \( \Xi \ / \text{Å} \) | 249 \( \pm \)2.4 |
| \( \alpha \) | 2.7 \( \pm \)0.2 | \( I_{\text{cyl}}(0) \ / \text{cm}^{-1} \) | 0.95 \( \pm \)0.01 |
| \( Q_c \ / \text{Å}^{-1} \) | 0.024 \( \pm \)0.004 | Cylinder radius, \( r \ / \text{Å} \) | 4.9 \( \pm \)0.3 |
| \( R_m \ / \text{Å} \) | 258 \( \pm \)60 | Cylinder length, \( L \ / \text{Å} \) | \(~190\) |

#The parameters of power law exponent \( \alpha \) and the crossover point \( Q_c \) obtained from the scattering curve. The mesh size, \( R_m \), was calculated by \( R_m = 2\pi/Q_c \). The other parameters obtained from the fitting results by equations 3.1 and 3.3.

In Figure 3.8, a typical XRD spectrum of pure dry gelatin films displays three main peaks, at \( 2\theta = 7.6^\circ, \sim 20^\circ \), and \( 31.9^\circ \). These peaks (yellow circle marked) depict the diameter of the triple helix (repeat distance \( d \) of 11.7 Å), the amorphous halo, and the
amino acid contacts along the axis of single helices (repeat distance d of 2.8 Å), respectively. Thereby a typical diameter of the gelatin helix is about 10 Å (D = 0.886*d) which is highly consistent with the SANS and the results in Figure 3.7.

Figure 3.7 visualizes the structure of the gelatin gels in the broad range from nano to micrometer scale as obtained from the SANS-VSANS data. Gelatin chains in a gel network show two conformations on a local scale, namely segments of random coils and of triple-helical. On a macroscopic scale, triple-helices are not homogeneously distributed in the gel. Large clusters with dense triple-helical segments (dense phase) are detected by SANS-VSANS at the low Q-range. The dilute gelatin phase shows a spatially confined cavity structure with an average mesh size of ~260 Å appearing as homogeneously distributed meshes throughout the gel matrix and which allows the ion transport and magnetite particles to form.

### 3.3.3 The Structure as a Function of Gelatin Concentration

From the phase diagram discussed in the former part, we know that gelatin has general properties as a function of concentration. In this part, I would like to investigate the effect of gelatin concentration on the microstructure.
Figure 3.9. SANS scattered intensity versus scattering vector Q for gelatin in D\textsubscript{2}O as a function of gelatin concentration (T = 20 ±2°C). The scattering cross section was normalized with gel volume fraction $\phi$. At low Q ($< 0.002$ Å\textsuperscript{-1}), VSANS data are also presented after rescaling. The solid lines represent a fit by equation 3.1 and 3.4.

SANS data from hydrogels of varying gelatin concentration between 6 and 30 wt\% are shown in Figure 3.9. The scattering was normalized with the gel volume fraction $\phi$. The scattering below $Q \sim 0.01$ Å\textsuperscript{-1} (regime I) show an accumulation of a network structure of similar scattering behavior. In the high Q-regime, we observe with increasing gel concentration a lowering of the power law exponent from $\sim$2 to $\sim$1. The trend to exponent $\beta = 1$, i.e. scattering from rod-like particles, indicates an enhanced amount of triple helix bundles, which is not accompanied by a significant change of average mesh size (Table 3.2). The power law $\beta = 2$ means a Gaussian chain conformation. Thus, there is a conformation change accompanied by varying the gelatin concentration.

In order to fit the data with different conformation in high Q, the scattering can be well described by the solid lines representing the best fit of the following empirical functional model developed by B. Hammouda et al\textsuperscript{104}:

$$\frac{d\Sigma}{d\Omega}(Q) = \frac{A}{Q^n} + \frac{C}{1+(Q\xi)^m} + BKG$$  \hspace{1cm} (3.4)
The correlation length at high-\(Q\), \(\xi\), represents the weighted-average inter-distance between the gelatin triple helices, also seen as the inter-fibrillar crosslinking distance like spacing in cryo-TEM micrographs.\(^{139}\) In the above equation 3.4, a scattering of the gelatin network is described by the first term, \(A/Q^n\) and is qualitatively similar to Porod-like scattering which usually was used to evaluate the clustering strength of the primary scattering objects. Scattering at larger \(Q\) in regime II is expressed by the second term \(C/[1 + (Q\xi)^m]\) and has been used to characterize the polymer/solvent interaction and chain solvation characteristics.\(^{104}\) In this thesis, it identifies the gelatin morphology and the network structure on the nanoscale. The amplitudes of the Porod and Lorentzian terms (\(A\) and \(C\), respectively), the \(Q\) independent incoherent background scattering (BKG), and the Porod and Lorentzian scattering exponents (\(n\) and \(m\), respectively) were obtained by a nonlinear least-squares fit of the data.

Scattering data were fitted with the correlation length model as well as the Beaucage equation (in both regimes of the large and small structure), and the extracted parameters are listed in Table 3.2, respectively.

**Table 3.2.** Parameters of Gelatin hydrogels in D\(_2\)O obtained from the SANS/VSANS data.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>6wt% / D(_2)O</th>
<th>12wt% / D(_2)O</th>
<th>18wt% / D(_2)O</th>
<th>30wt% / D(_2)O</th>
</tr>
</thead>
<tbody>
<tr>
<td>(\frac{d\Sigma}{d\Omega}(0)) / cm(^{-1})</td>
<td>1050 ±32</td>
<td>2150 ±46</td>
<td>2500 ±50</td>
<td>5300 ±73</td>
</tr>
<tr>
<td>(R_g) / Å (Regime I)</td>
<td>1114 ±32</td>
<td>1576 ±37</td>
<td>1590 ±30</td>
<td>1704 ±22</td>
</tr>
<tr>
<td>(\alpha)</td>
<td>2.5 ±0.2</td>
<td>2.6 ±0.2</td>
<td>2.7 ±0.2</td>
<td>2.7 ±0.1</td>
</tr>
<tr>
<td>(Q_c) / Å(^{-1})</td>
<td>0.0236 ±0.004</td>
<td>0.0225 ±0.005</td>
<td>0.024 ±0.004</td>
<td>0.0295 ±0.005</td>
</tr>
<tr>
<td>(R_m) / Å</td>
<td>268 ±51</td>
<td>279 ±70</td>
<td>258 ±60</td>
<td>213 ±29</td>
</tr>
<tr>
<td>(R_g) / Å (Regime II)</td>
<td>43.7 ±0.2</td>
<td>46.2 ±0.1</td>
<td>75.2 ±0.8</td>
<td>78.0 ±0.6</td>
</tr>
<tr>
<td>(C)</td>
<td>1.65 ±0.25</td>
<td>1.06 ±0.024</td>
<td>0.680 ±0.0092</td>
<td>0.255 ±0.0022</td>
</tr>
<tr>
<td>(m)</td>
<td>1.63 ±0.01</td>
<td>1.63 ±0.01</td>
<td>1.49 ±0.01</td>
<td>1.72 ±0.02</td>
</tr>
<tr>
<td>(A/Q^n) ((Q = 0.02) Å(^{-1}))</td>
<td>0.44</td>
<td>0.58</td>
<td>0.76</td>
<td>1.1</td>
</tr>
<tr>
<td>(\xi) / Å</td>
<td>78 ±8</td>
<td>41 ±1</td>
<td>21 ±0.5</td>
<td>10.2 ±0.1</td>
</tr>
<tr>
<td>(\beta)</td>
<td>1.7</td>
<td>1.5</td>
<td>1.2</td>
<td>0.98</td>
</tr>
<tr>
<td>BKG / cm(^{-1})</td>
<td>0.063</td>
<td>0.074</td>
<td>0.090</td>
<td>0.16</td>
</tr>
</tbody>
</table>

#The parameters of power law slope \(\alpha\), \(\beta\) and the crossover point \(Q_c\), obtained from the scattering curve. The mesh size, \(R_m\), was calculated by \(R_m = 2\pi/Q_c\). The other parameters were obtained from the fitting results by equations 3.1 and 3.4.
Table 3.2 compares the concentration dependence of the low-Q larger cluster radius of gyration of $R_g$ and the high-Q polymer chain correlation length $\xi$ for the gelatin samples at room temperature.

The radius of gyration slightly increases with the gelatin concentration from ~1114 Å to ~1704 Å. The scattering intensity at zero angle, $d\Sigma/d\Omega(0)$, increasing linearly with the gelatin concentration indicates that the number density of the large gelatin clusters correlates with the gel concentration. Figure 3.10 visualizes the two structures of the gelatin gels changing with the increase of the gelatin concentration which was obtained from the SANS data. The 3D reconstructed models of the large clusters change their shape from partially folded protein-like structure to a surface rough particle-like structure. The results consist with the power law where $\alpha$ in regime I increases with increasing gelatin concentration, from 2.5 to 3.4 for 6 to 30 wt% gelatin, respectively. Due to the swelling effect on the large clusters. This implies that the gelatin network consists of large phase-separated domains (collapsed polymer coils) with a surface fractal conformation when concentrating the hydrogels.

![Figure 3.10](image)

**Figure 3.10.** Schematic representation of gelatin structure changes with the concentration: the larger cluster reconstruction from the scattering data in regime I by a rapid *ab initio* shape determination program DAMMIF of (A) Gelatin 6 wt% in D$_2$O, (B) Gelatin 18 wt% in D$_2$O and (C) Gelatin 30 wt% in D$_2$O. Models of high Q small structure of gelatin molecules conformation changes from (D) Gelatin random coils to (E) Partly folded gelatin triple helices, and then (F) Gelatin triple-helices.

From Table 3.2, the correlation length $\xi$ significantly decreases with gelatin concentration from 80 to 10 Å. An increase in the compactness of the network with concentration is related to an increase in the number of interaction points (Hydrogen bonding interactions) and an associated reduction in the inter-chain (random coils) distance at these
points. Moreover, the crossover between the two Q regimes at $Q_c$ of $\sim 0.024 \, \text{Å}^{-1}$ (sample Gelatin 18 wt% in D$_2$O) allows us to estimate the gelatin average 3D cage size of $R_m = 2\pi/Q_c \approx 258 \, \text{Å}^{-1}$. The average cage size of different samples seems not accompanied by a significant change with the increasing of the gelatin concentration. However, higher concentration samples show the smaller value of $R_m = 213 \, (\pm 29) \, \text{Å}^{-1}$ for gelatin 30 wt% when comparing to gelatin 6 wt% of $R_m = 268 \, (\pm 51) \, \text{Å}^{-1}$. The power law exponent $\beta$ in the regime II seems to be related with the gel concentration and is decreased from 1.7 to 0.98 when the gel is concentrated. The trend to $\beta = 1$, i.e. scattering from a rigid rod-like particles, indicate an enhanced amount of triple helix bundles.

The clustering strength ($A/Q^n$) for the regime II data is listed for the concentration of gelatin samples in D$_2$O in Table 3.2. High clustering strength corresponds to networks (where both chain-ends stick to other chains) while low clustering strength corresponds to dissolved chains. The intermediate case corresponds to branched structures. Thus, the higher concentration gelatin samples show network features (e.g. gelatin 30 wt% of 1.1) while lower concentration gelatin samples show a branched structure with partially dissolved chains in solution (e.g. gelatin 6 wt% of 0.44). Clustering is seen to increase while solvation decreases with increasing of the gelatin concentration. These two trends are opposite pointing to different driving forces for these two phenomena.

When concentrating the sample to a very dry state, the structure of the gelatin becomes glass-like on the right side of the phase diagram (Figure 3.5). The dry sample is very dense in which gelatin molecules are mostly in triple-helix conformation and closely packed in large fiber-like crystals. In Figure 3.11 a result from SANS on the dry gelatin thin films sample was presented. The scattering curve can be divided into two parts. The scattering below 0.01 $\, \text{Å}^{-1}$ follows power law scattering with an exponent of $\sim 3$ indicating a mass fractal structure (fractal of the large open pores). The scattering above 0.01 $\, \text{Å}^{-1}$ shows flat scattering due to the densely packed gelatin triple-helices. The result consists with an AFM phase image (inset of Figure 3.11) which shows large open pores (in a dark area) with of typical cage size of 500 Å. The big domains (in bright color) are composed of crystallized gelatin triple helices.
Figure 3.11. SANS scattered intensity versus scattering vector Q for gelatin dry films. The solid line represents a power law fitting. The inset is an AFM result on a dry gel and a schematic representation of the gelatin structure.

Figure 3.12 shows the SANS curves from gelatin of various concentrations increasing from 0.1 wt% to 1 wt% in acetate-acetic acid buffer solutions (in D$_2$O, 100 mM, pH = 5.1). The temperature was fixed at T = 20°C of which temperature all samples are in the solution phase. Scattering profiles covered two decades with a lower Q power law exponent $\alpha$ of ~2.9 and a higher Q of $\beta$ of ~1.6 indicating a typical polymer network (see Table 2.2). The Q range follows the $\beta \sim 1.6$ power law exponent which corresponds to the Flory exponent $\nu = 3/5$ ($\nu \equiv 1/\beta$). With an increase in gelatin concentration, the exponent $\beta$ decreases toward 1.43 indicating a less swollen behavior. Thus, the high Q scattering of samples 0.1 wt%, 0.2 wt% and 0.3 wt% show the characteristics of swollen Gaussian chains in a good solvent while above 0.5 wt% of gelatin concentration indicate a badly swollen chain in a theta condition. Moreover, all data were fitted with the correlation length model, and the extracted parameters listed in Table 3.2.
Figure 3.12. SANS scattered intensity versus scattering vector \( Q \) for gelatin solutions from 0.1 wt% to 1 wt% in acetate-acetic acid buffer in D\(_2\)O, 100 mM, pD = 5.3. The solid lines represent the best fitting by equation 3.4.

In the phase diagram (Figure 3.5.), the gelatin overlap concentration \( c^* \) is close to 0.5 wt%, where the correlation length \( \xi \) has a minimum value of 7.8 ±1.2 Å. With increasing concentrations in the semi-dilute range from 0.2 wt% of gelatin to \( c^* \), the correlation length decreases according to a reduction in the inter-chain (random coils) distance. In contrast, the \( \xi \) goes up for the 1 wt% of gelatin, which may be caused by a partial transition of a flexible (random coils) to a stiff chain (gelatin triple-helices) conformation decreasing the intra-chain physical ‘crosslink points’ (i.e. the hydrogen bonding). Further, the clustering strength \( A/Q^0 \) is increased with increasing the concentration from 0.2 wt% reflecting a gelation trend.

From the above discussion, we can well describe the gel structure and represent it in Figure 3.7 and Figure 3.10. Gelatin chains in a gel network show two conformations on a local scale, namely segments of random and triple-helical coils. On a macroscopic scale, triple-helices are not homogeneously distributed in the gel. Large clusters with an excess concentration of triple-helical segments (dense phase) are detected by SANS-
VSANS at the low Q-range. The dilute gelatin phase constructed by triple helical coils and random coils shows a spatially confined cavity structure with average cage size of about 250 Å appearing as homogeneously distributed cages throughout the gel matrix and which allows the ion transport and magnetite particles to form. With an increasing of the volume fraction in a semi-dilute gelatin solution, the clustering strength increases as the force for gelatin gelation accompanied with a conformation transition from random coils to triple helices. Further increasing the concentration to a dry gelatin state, densely packed gelatin triple helix crystals form with a small amount of the large fractal pores.

Table 3.3. Parameters of Gelatin solutions (with acetate buffer, pD≈5.2) in D₂O obtained from the SANS data.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>0.1 wt%</th>
<th>0.2 wt%</th>
<th>0.3 wt%</th>
<th>0.5 wt%</th>
<th>1.0 wt%</th>
</tr>
</thead>
<tbody>
<tr>
<td>α</td>
<td>2.9</td>
<td>3</td>
<td>2.9</td>
<td>2.8</td>
<td>2.8</td>
</tr>
<tr>
<td>A/Q²((\Omega = 0.02 ) Å⁻¹)</td>
<td>0.0138</td>
<td>0.0097</td>
<td>0.0066</td>
<td>0.102</td>
<td>0.183</td>
</tr>
<tr>
<td>ξ/Å</td>
<td>37.8±11</td>
<td>40±5</td>
<td>25.4±2.7</td>
<td>7.8±1.2</td>
<td>11.8±3</td>
</tr>
<tr>
<td>β</td>
<td>1.65</td>
<td>1.56</td>
<td>1.54</td>
<td>1.46</td>
<td>1.44</td>
</tr>
<tr>
<td>BKG/cm⁻³</td>
<td>0.036</td>
<td>0.037</td>
<td>0.037</td>
<td>0.038</td>
<td>0.039</td>
</tr>
</tbody>
</table>

# The parameter β obtained from the SANS data directly; the other parameters obtained from the fitting results of equations 3.4.

3.3.4 The Contrast in Gelatin Hydrogels

An important advantage of SANS is the possibility to vary the scattering contrasts by the variation of heavy water content in H₂O/D₂O mixture. The contrast variation method allows to match (hide, make invisible) a part of a structure in a multicomponent material.¹⁴³-¹⁴⁷ In this part, the scattering length density of gelatin in the hydrogel phase at the different length scales is investigated by the contrast variation method.

The scattering cross-sections of the 18wt% gelatin hydrogels for eight H₂O/D₂O concentrations are shown in Figure 3.13. The scattering curve of gelatin in D₂O shows the maximal contrast in comparison to others within the measured Q-range. It seems that the scattering in the regime I and regime II have different minima in various samples with different D₂O volume fraction. That means inhomogeneous contrast difference in the large and small structure in the hydrogels. Thereby different structures are found in
the detected length scales which is consistent with the former SANS results of the two level structure as discussed above.

![Figure 3.13](image)

**Figure 3.13.** The scattering cross-sections measured by SANS of the 18wt% gelatin hydrogels for several H₂O/D₂O concentrations (T = 20 ± 2°C).

In order to understand the contrast measurement results, scattering intensity at two Q values of 0.006 Å⁻¹ in regime I and 0.054 Å⁻¹ in regime II, as well as the incoherent background are plotted as a function of D₂O volume fraction in Figure 3.14. Moreover, scattering data were fitted with the correlation length model and the extracted parameters listed in Table 3.3.

In Table 3.4 the incoherent background of various samples is linearly decreased with the increasing of the D₂O volume fraction indicating an accuracy control on the solvent component of D₂O. It is allowed to carefully subtract the background and to extract the contrast variation results. In Figure 3.14 (B), the correlation length ξ obtained from the fitting results has no significant changes except two maximum points around 45 v/v% of D₂O. The maximum correlates with the intensity minimum in Figure 3.14 (C), where the scattering at the large Q regime corresponding to the gelatin molecule structure is almost matched. The contrast matching at nearly 45 v/v% of D₂O indicates a similar contrast with protein since gelatin is a mixture of polypeptides and proteins produced by
partial hydrolysis of collagen. As the scattering curves of 18wt% gelatin solution within Region II follow the scaling low close to $Q^{-1}$, we could conclude that at 45% of heavy water gelatin helices are matched. Thereby, the scattering at the matching point shows two quite distinctive correlation lengths which is reasonable (the small structure is matching thus the large structure is shown). In Figure 3.14 (B), the scattering intensity at $Q = 0.006 \text{ Å}^{-1}$ has a minimum at around 25 v/v% of D$_2$O that is a large shift from the gelatin molecule matching point indicating some different structure. From the discussion above, scattering in Regime I corresponds to larger clusters, which have a dense gelatin core and a rough surface. The core is quite dense/crystalline that may still have some bound H$_2$O not being exchangeable with the solvent of the D$_2$O/H$_2$O mixture.

![Figure 3.14](image)

**Figure 3.14.** Parameters obtained from SANS contrast measurements: (A) Correlation length from fitting results by equation 3.3. (B) SANS scattering cross section in Regime I at $Q = 0.006 \text{ Å}^{-1}$. (C) SANS Scattering cross section in Regime I at $Q = 0.054 \text{ Å}^{-1}$. (D) Sum of SANS Scattering intensity in the $Q$-range from $Q = 0.005$ to $0.05 \text{ Å}^{-1}$.

Thermogravimetry (TG) was performed on freeze-dried gelatin samples in a N$_2$ atmosphere in order to identify any thermal event. The TG plot of pure gelatin normally dis-
plays three thermal stages, i.e. loss of free and bound water, between 25 and 250 °C, gelatin decomposition, between 250 and 450 °C, and finally combustion of the remaining material, between 450 and 750 °C. In Figure 3.14, the gelatin sample displayed a 5 wt% mass loss in a temperature range of 25-100 °C and 10 wt.-% mass loss in a temperature range of 100-250 °C. The results indicate that in a freeze-dried gelatin sample contains ~5 wt% free water and ~5 wt% bound water. This may be the main reason that the large clusters have a very different contrast. The large clusters are very dense and could be considered as insoluble gelatin crystalline particles containing water (H₂O).

Further, summarized intensity shows that around 28 v/v% of D₂O samples have a minimum with a very wide distribution. The minimum is close to the large clusters of the 18 wt% gelatin (25 v/v% of D₂O) indicating the large cluster scattering having the largest contribution on the hydrogels scattering in the measured Q range.

**Table 3.4.** Parameters of Gelatin hydrogels in D₂O/H₂O mixture obtained from SANS contrast measurements.

<table>
<thead>
<tr>
<th>D₂O/H₂O V/V%</th>
<th>BKG / cm⁻¹</th>
<th>ξ / Å</th>
<th>I(Q=0.006 Å⁻¹)</th>
<th>I(Q=0.0543 Å⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>100 (In D₂O)</td>
<td>0.10</td>
<td>20.8 ±0.5</td>
<td>36.1986</td>
<td>0.23595</td>
</tr>
<tr>
<td>70</td>
<td>0.308</td>
<td>12.9 ±1</td>
<td>15.1373</td>
<td>0.04422</td>
</tr>
<tr>
<td>50</td>
<td>0.464</td>
<td>101 ±6</td>
<td>6.26237</td>
<td>0.01319</td>
</tr>
<tr>
<td>45</td>
<td>0.494</td>
<td>109 ±3</td>
<td>4.49362</td>
<td>0.0093</td>
</tr>
<tr>
<td>40</td>
<td>0.556</td>
<td>6.4 ±1</td>
<td>3.55307</td>
<td>0.01178</td>
</tr>
<tr>
<td>35</td>
<td>0.600</td>
<td>8.3 ±0.5</td>
<td>2.19312</td>
<td>0.02448</td>
</tr>
<tr>
<td>30</td>
<td>0.666</td>
<td>11.6 ±0.6</td>
<td>1.82803</td>
<td>0.04637</td>
</tr>
<tr>
<td>28</td>
<td>0.622</td>
<td>15.9 ±0.6</td>
<td>1.18066</td>
<td>0.05106</td>
</tr>
<tr>
<td>25</td>
<td>0.744</td>
<td>13.6 ±1</td>
<td>0.99851</td>
<td>0.07334</td>
</tr>
<tr>
<td>20</td>
<td>0.742</td>
<td>24.8 ±2</td>
<td>1.41948</td>
<td>0.084</td>
</tr>
<tr>
<td>10</td>
<td>0.863</td>
<td>19.3 ±1</td>
<td>1.25984</td>
<td>0.14856</td>
</tr>
<tr>
<td>0 (In H₂O)</td>
<td>0.932</td>
<td>21.8 ±0.5</td>
<td>2.9888</td>
<td>0.22914</td>
</tr>
</tbody>
</table>

# The correlation length (ξ) and the incoherent background (BKG) obtained from the fitting results of Equation 3.4. The other values are obtained from the scattering curve.

Thus, gelatin chains in hydrogels could be found in three configurations, namely chain-like, helix-like, and in crystalline-like conformation. The contrast matching results are related to the structure/conformation of the gelatin. In later chapters we will discuss mineralization of the magnetite particles in different gelatin matrixes, namely in 6 and
18 wt% of gelatin in water. So it was necessary to proceed with the contrast variation study of the 6 wt% hydrogels; as we will show next it is different from the 18 wt% gelatin result.

The scattering contrast measurement data of 6wt% gelatin solution were obtained only in the intermediate Q-range, and are shown in Figure 3.16 (A). The scattering curve of gelatin in D$_2$O has a maximum in all of the measured Q range similar to the 18 wt% results. In order to analyse the contrast measurement results, scattering intensity at different Q values is summarized in the Q range between 0.005 and 0.05 Å and plotted vs. Q and as a function of D$_2$O volume fraction in Figure 3.16 (B) and (C). It seems that a minimum scattering can be found at the D$_2$O volume fraction around 28 v/v%. Further summarized intensity shows that around 28 v/v% of D$_2$O samples have a minimum with a very wide distribution. The minimum is close to the large clusters of the 18 wt% gelatin indicating the large cluster scattering having the largest contribution on the hydrogels scattering in the measured Q range. Thus, in the work, 28 v/v% of D$_2$O will be used as the matching point for matching the scattering of gelatin in the organic-inorganic composites.

Therefore, in case of 6wt% the contrast of the large particles in Range I and individual chains in Range II show approximately the same contrast matching condition at about 28% of the heavy water content. That means the most of the chains are in polymer-like conformation.
Figure 3.16. (A) SANS scattering curves for 6 wt% gelatin in D$_2$O/H$_2$O mixtures (T = 20 ±2°C); (B) scattering intensity at different Q for samples in a mixture of D$_2$O/H$_2$O; (C) sum of scattering intensity in the Q-range from Q = 0.005 to 0.05 Å$^{-1}$ as function of heavy water content in D$_2$O/H$_2$O mixture.

3.3.5 The Temperature Effects

The properties of gelatin hydrogels are very sensitive to temperature variations because of their special network structure. The thermally induced changes of properties (such as the viscosity, the phase, loss modulus, etc.), are mainly caused by segments of...
gelatin chains (random coils) organizing intra-molecularly into the collagen fold (α-helix). In the concentrated hydrogels, the intermolecular interaction of helices forms physical “crosslinks” such as hydrogen bonds, hydrophobic interactions.116, 131, 142 In this part, we want to use small angle scattering to probe the structure change as the function of temperature.

From the former scattering results, we know that the hydrogels have two levels of structure, the dense phase of large gelatin clusters (with mainly dense gelatin helices) and the dilute phase of triple-helices and random coils. The two level structure shows the main scattering contribution in each Q regime.

Figure 3.17. SANS-VSANS scattered intensity versus scattering vector Q (Regime I, low Q for large structure) for gelatin 18 wt% in D$_2$O at different temperatures.

Figure 3.17 shows the effects of temperature on the scattering profiles (Regime I) of 18 wt% gelatin in D$_2$O in the hydrogels (equal or below 20°C) or aqueous solutions (above 30°C). The corresponding scattering related to the large cluster structure have no significant changes in the temperature below 90°C indicating a stable structure in the temperature range from 4 °C to 60°C, the temperature range in which we are performing the experiment. The temperature at 90°C shows a dramatical decrease the scattering by a factor of 3. The changes may be caused either by the dense-dilute phase transition or
exchanging of the bound H₂O with D₂O at such temperature (change the contrast). Thus, the large structure is stable below 60°C.

**Figure 3.18.** Log-linear plot of SAXS scattered intensity versus scattering vector Q (Regime II, Large Q for small structure) for gelatin 18 wt% in D₂O at different temperatures. The inset picture is the SAXS intensity at Q = 0.01444 Å⁻¹ as a function of temperature.

Figure 3.18 displays the influences of temperature on the SAXS scattering profiles (Regime II, the network structure) of 18 wt% gelatin in D₂O including a gel to sol transition process. There are various changes in the different Q ranges. A key Q value of 0.478 Å⁻¹ corresponding to d=2π/Q ≈ 13 Å is d-space of the inter-gelatin helices, where in the dry state of gelatin the triple-helix peaks are located at around 12 Å from the former XRD measurement. Thus, the key Q values reflect the gelatin triple-helix changes in the gelatin solutions or gels. Inset of Figure 3.18 shows a decreasing of scattering intensity at Q = 0.01444 Å⁻¹ with increasing the system temperature. The gelatin triple-helices mainly cause the change to random coils/α-helix transition in which triple-helices have a much higher scattering than the coils. Thus, from the SAXS results, in the temperature range from 4°C to 50°C, there is a continuous loss of gelatin triple-helix conformation in the systems. The results consist with thermal and rheology experimental results discussed before.
Figure 3.19. Parameters from SAXS scattered intensity as a function of temperature for gelatin 18 wt % in D$_2$O at different Q.

In order to demonstrate the temperature effects on the structural transformations and properties of gelatin, scattering intensity at different Q values as well as the summarized intensity (Q range of 0.0478 Å$^{-1}$ to 0.0056 Å$^{-1}$) were plotted as a function of temperature in Figure 3.19 (A), (B), (C), and (D). Figure 3.19 (B) and (C) have similar changes as the triple-helix to coil transition in the whole temperature range. The intensity decreases sharply during the gel-sol transition temperature while smoothly changing in the high temperature range above 40°C and below 20 °C. Results indicate that the phase transition mainly happens in the temperature between 20°C and 40 °C.

Figure 3.19 (A) shows a peak with a maximum at around 37°C. In this Q range, the scattering reflects the gelatin inter-chain interaction. During the gel to sol transition the system becomes quite complex. A microphase separation may make the scattering intensity higher due to large scattering object formation. Once the transition finished,
the clear phase separation decreases the scattering intensity. Moreover, the peak position is comparable with the DSC gel-sol mid temperature but with 7°C shift.

In Figure 3.19 (D), the scattering intensity increases with the temperature which is the opposite behavior to the results in Figure 3.19 (B) and (C) indicating increasing random coil ratios in the system. The changes of the scattering intensity are in good agreement with the intermediate Q range changes.

**Figure 3.20.** Parameters from SAXS scattered intensity as a function of temperature for gelatin 18 wt% in D₂O. (A) The correlation length (ξ) and (B) the clustering strength (A/Qⁿ, Q = 0.12 Å⁻¹) obtained by fitting with equation 3.4. (C) The radius gyration (Rᵋ) and (D) the high Q power law exponent (β) obtained by fitting with equation 3.1.

The SAXS scattering data (Figure 3.18) were fitted with the correlation length model as well as the Beaucage equation and the extracted parameters are plotted in Figure 3.20, respectively. The inter- and intra-chain correlation length ξ (Figure 3.20 A) as a function of temperature has a minimum at 37°C indicating that the thermally induced reorganization process dramatically increases the physical crosslinks and reduces the
inter- and intra-chain distance. A microphase separation during thermal transition results in a $R_g$ maximum (Figure 3.20 C). In Figure 3.18 B, the clustering strength decreasing with the increase of the temperature which as the main parameter influence on the triple-helix to coil transition. The high Q power law exponent increasing from about 1 to 1.6 indicates a shape conformation transition from rod-like particles to swollen chains in good solvents (Figure 3.20 D). That implies a gelatin conformation transition from triple-helices to coils.

From the SAXS scattering results, the temperature effects on the gelatin structure are concluded. At a temperature below 60°C there is nearly no change in the large cluster structure. The gel network structure is changing because of inter- and intra-chain interaction and a triple-helix to coil transition. The SAXS results also show that decreasing clustering strength is correlated with the conformation transition. During a phase transition in the phase diagrams, the intermediate regime shows a microphase separation with large influence on the correlation length and $R_g$.

### 3.3.6 The pH Effects

There are two types of gelatin. Type A, with the isoelectric point (IEP) of 8 to 9, is derived from collagen with acid pretreatment. Type B, with IEP of 4.8 to 5.2, is the product of an alkaline pretreatment. In this work, all the gelatin used is Type B gelatin. The pH of the gelatin (Type B) hydrogels is about 5 slightly varying with the concentration. At pH $\approx 5$, the viscosity of Type B gelatin is minimal and the gel strength is maximal which reflects the importance of pH for rheological properties and industrial applications. When the pH is close to the isoelectric point (IEP), gelatin is neutral in charge. If the pH is higher than the IEP, the gelatin is negatively charged while at a lower pH gelatin becomes positively charged. Here we want use SANS-VSANS to study the microstructure changes accompanied by the pH changes.

Figure 3.21 shows the effects of pD on the SANS-VSANS scattering profiles of gelatin hydrogels with pD around 5.3 (in D$_2$O), 3.7 (in 0.1 M DCl), and 11.4 (in 0.1 M NaOD). The pD of a solution in D$_2$O is the pH meter reading (pH) plus 0.4. In the regime II (Figure 3.21), the SANS results indicate the same level scattering intensity in 0.1M DCl comparsion to the samples in D$_2$O (pD $\approx 5.3$). The scattering of large structures found in regime-I has increase the size between the two samples (from 1590 ±30 Å of gelatin in
D$_2$O to 2096 ±61 of gelatin 0.1M DCl, Table 3.5). Moreover, there is nearly no change of the crossover point, $Q_c$, indicating the same cage size in the two samples. Figure 3.22 displays the effects of higher pD on the SANS-VSANS scattering profiles of gelatin hydrogels with pD around 11.4 (gelatin in 0.1 M NaOD). Scattering results show that significant increasing the low $Q$ power law exponent from almost 0 (guinier scattering) to ~ 3 (fractal structure, see the inset of Figure 3.22; as a result of higher clustering strength factor, see Table 3.5) revealing aggregation of large clusters in the sample with higher pD. In the regime II, high $Q$ scattering has lower scattering intensity in the higher pD sample indicating lower of triple helices in the hydrogels.

![Figure 3.21](Figure 3.21. SANS-VSANS scattering intensity versus scattering vector $Q$ for gelatin 18 wt % in D$_2$O and D$_2$O with 0.1 M DCl. The solid lines represent the fitting by Beaucage equation of equation 3.1 ($Q < 0.01$ Å$^{-1}$) and correlation length model of equation 3.4 ($Q > 0.01$ Å$^{-1}$).

Fitting parameters from SANS-VSANS results in Table 3.5 show that the correlation length decreases from 25 Å of the sample in D$_2$O to 13.8 Å in an acid condition and
11.5 Å in a base condition. The changing correlated with the changing of parameter C which reflects decreasing of the triple helices amount in the sample pH far away from the IEP. In gelatin, water molecules are placed in interstitial positions and stabilize the triple-helices by two noncovalent interactions: hydrogen bonding and electrostatic interactions. As pH deviated from the isoelectric point, polar groups such as -COOH, -NH₂ partly transform to their charged groups –COO⁻ (pH > IEP) and –NH₃⁺ (pH < IEP). Those changes has broken the interaction between gelatin residues thus influencing on the stability of gelatin triple helices. So either in much higher pH or in much lower pH than the IEP, gelatin hydrogels have less triple helix conformation thus owing poor mechanical properties.

**Figure 3.22.** SANS-VSANS scattered intensity versus scattering vector Q for gelatin 18 wt% in D₂O and D₂O with NaOD 0.1 M. The solid lines represent the fitting by Beaucage equation of equation 3.1 (Q < 0.01 Å⁻¹) and correlation length model of equation 3.4 (Q > 0.01 Å⁻¹).
Table 3.5. Parameters of Gelatin hydrogels in D\textsubscript{2}O with different pD values obtained from the SANS-VSANS data.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Gelatin18 wt% / D\textsubscript{2}O (Gel pD ≈ 5.3)</th>
<th>Gel + 0.1 M DCl pD ≈ 3.7</th>
<th>Gel + 0.1 M NaOD pD ≈ 11.4</th>
</tr>
</thead>
<tbody>
<tr>
<td>R\textsubscript{g} (Q regime I) / Å</td>
<td>1590 ± 30</td>
<td>2096 ± 61</td>
<td>Aggregate</td>
</tr>
<tr>
<td>R\textsubscript{g} (Q regime II) / Å</td>
<td>75.2 ± 0.5</td>
<td>13.8 ± 1</td>
<td>11.5 ± 1</td>
</tr>
<tr>
<td>Correlation length, ξ / Å</td>
<td>21 ± 0.5</td>
<td>1.2 ± 0.1</td>
<td>1.1 ± 0.2</td>
</tr>
<tr>
<td>High Q power law slope, β</td>
<td>1.2</td>
<td>0.82</td>
<td>0.89</td>
</tr>
<tr>
<td>Clustering strength A/Q\textsuperscript{a} (Q = 0.02 Å\textsuperscript{-1})</td>
<td>0.76</td>
<td>0.33</td>
<td>0.23</td>
</tr>
<tr>
<td>Lorentz coefficients, C</td>
<td>1.65</td>
<td>2096 ± 61</td>
<td>Aggregate</td>
</tr>
</tbody>
</table>

# The parameters obtained from the fitting results of equations 3.1 and 3.4.

### 3.3.7 Salt Effects

The gelatin hydrogels are composed of physically interconnected triple-helices and random coils, which are stabilized by intermolecular hydrogen bonding, hydrophobic bonding, and electrostatic interaction.\textsuperscript{114} Ions of a salt could affect the gelatin structure via changing the electrostatic interaction strength, the formation of salt bridges and hydration and hydrogen bonding.\textsuperscript{148} In this part, we want to show the effect of salts on the hydrogel structure by using small angle scattering methods.

In Figure 3.23 the influences of salts on the SANS-VSANS scattering profiles of 18 wt% gelatin in D\textsubscript{2}O is shown in the presence of different salts. Chloride salts with cations of different valency, namely monovalent (Na\textsuperscript{+}), divalent (Ca\textsuperscript{2+}, Cu\textsuperscript{2+}, Co\textsuperscript{2+}), and trivalent (Fe\textsuperscript{3+}), of different concentrations were used. The scattering profiles were fitted both with the Beaucage and correlation length model in regimes I and II correspondly. Extracted parameters are listed in Table 3.6.

In Figure 3.23, the forward scattering dΣ/dΩ(0) of larger clusters in regime I is increased after the addition of salts with monovalent and divalent cations in comparison with the sample in D\textsubscript{2}O. Low Q scattering results show that the addition of a salt affects the structure without dramatically increasing the size but increasing the number density and/or contrast of the larger clusters. All monovalent and divalent cations have no significant effect on the scattering intensity as well in the regime II. A possible reason for that is that the addition of large amounts of salts to macromolecular systems (e.g.
protein, biopolymers) causes precipitation of the dispersed substance, thus increasing the dense large cluster phase in the hydrogels. That is the so-called salting out behavior. The ionic strength for all samples reaches a threshold value that for all samples seems to have universal influence on the larger structure. Trivalent cation, Fe$^{3+}$ indicates a strong effect on aggregation of large gelatin clusters as shown for the lower Q scattering (power law exponent of 3).

**Figure 3.23.** SANS-VSANS scattered intensity versus scattering vector Q for gelatin 18 wt% in D$_2$O and salt solutions with different concentration and counterion valence. The inset represents the scattering intensity of samples at Q = 0.05 Å$^{-1}$.

In Figure 3.23 of regime II, the scattering of the gelatin network shows various changes depending on the species and concentration of salts. The addition of monovalent ions Na$^+$ in the hydrogels does not show clear changes in the scattering compared to the salt-free gels. There is only a slight decrease of the $R_g$ and correlation length $\xi$. However, the results difference between the sample in 0.1 M NaCl and 1 M NaCl (with different
Gelatin Hydrogel Matrices

... shows that the electrostatic interaction strength does not dominate the inter-helix interactions.

In the inset of Figure 3.23, we can see that the addition of divalent cations decreases the scattering intensity compared to the sample with Na\(^+\) and without additional salts. The \(R_g\) of the samples with divalent ions becomes smaller and is around 2/3 of the salt-free hydrogels. This result correlates with the smaller 3D-cage size of gelatin network. The correlation length of the calcium ion loaded hydrogels becomes nearly half of the salt-free gels. All those changes indicate that deformation happens in the divalent cation loaded hydrogels, where the gelatin triple-helices become shorter and less (helix to coil transition). From the other side, increasing of Ca\(^{2+}\) ions from 0.1 M to 1 M significantly decreases the clustering strength \(A/Q^n\). For the 1M salt concentration, the gelatin is in solution phase indicating that the ionic strength of Ca\(^{2+}\) ions has an influence on the gelation properties. Thus, the group of divalent cations has the ability to deform the gelatin triple-helix gel network in the applied salt concentration range.

Table 3.6. Parameters of Gelatin hydrogels in D\(_2\)O with different concentration and counterion valence obtained from the SANS-VSANS data.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>0</th>
<th>I</th>
<th>II</th>
<th>III</th>
</tr>
</thead>
<tbody>
<tr>
<td>(R_g/\text{Å})</td>
<td>75.2 ±0.5</td>
<td>60.4 ±31</td>
<td>65.8 ±1</td>
<td>46.9 ±16</td>
</tr>
<tr>
<td>(\xi/\text{Å})</td>
<td>21 ±0.5</td>
<td>16.8 ±6</td>
<td>16.8 ±0.1</td>
<td>12.4 ±0.9</td>
</tr>
<tr>
<td>(A/Q^n_{Q=0.02/\text{Å}^{-1}})</td>
<td>0.76</td>
<td>1.16</td>
<td>0.96</td>
<td>0.85</td>
</tr>
<tr>
<td>C</td>
<td>1.65</td>
<td>0.5</td>
<td>0.468</td>
<td>0.234</td>
</tr>
<tr>
<td>(Q_c/\text{Å}^{-1})</td>
<td>0.024</td>
<td>0.028</td>
<td>0.029</td>
<td>0.0294</td>
</tr>
<tr>
<td>(R_m/\text{Å})</td>
<td>262</td>
<td>224</td>
<td>217</td>
<td>214</td>
</tr>
</tbody>
</table>

# The parameters obtained from the fitting of SANS-VSANS results of equations 3.1 and 3.4.

The Fe\(^{3+}\) ions loaded into the hydrogel show significant increase of the scattering intensity in conjugation with the dramatrical increase of \(R_g\), clustering strength, and the gel 3D-cage size. The changes indicate a formation of more triple-helices, growing the helices length, and aggregation of the triple-helices (growing the rod-like species...
diameter). Thus, the Fe$^{3+}$ ions show a strong crosslinking effect on the gelatin network which are considered as junctions in between the gelatin inter-chains.

## 3.4 Summary

In this part, we have explored the structure of gelatin hydrogels on a large length scale by using small (SANS) and very-small (VSANS) angle neutron scattering in conjunction with small (SAXS) and wide (WAXS) angle X-ray scattering as well as rheology/thermal measurements.

The investigation of the gelatin hydrogels using SANS and VSANS revealed the existence of two phases (Figure 3.24): one is the colloid-like large clusters filled mainly with densely packed gelatin triple-helices (“dense phase”); the other is a 3D cage-like gel network composed of gelatin triple-helices and random coils (“dilute phase”). In the “dilute phase” the ratio between gelatin triple-helices and random coils is a function of gelatin concentration. The volume fraction of the “dense phase” is proportional to the gelatin concentration. The gel structure is well described by the multi-level model with next parameters: radius of gyration of the large clusters or the gelatin triple-helices $R_g$, the gelatin three dimensional cage size $R_m$, correlation length of the short range intra and inter-chain average distance $\xi$, the clustering strength $A/Q^0$ being a term to evaluate the aggregation strength. The gelatin structure was investigated as a function of gelatin concentration, ratios of $D_2O/H_2O$, temperature, pH, salt concentration and cation valence. Those experimental conditions influence the hydrogel properties via changing of the gel structures, e.g. the triple-helices to random coil ratios in the dilute phase, the aggregation of large clusters, length and diameter of the triple helices. A detailed study of the gelatin structure helped to choose appropriate gelatin concentrations, temperature, pH to achieve high efficiency for ion transport, optimal iron mineralization as well as high mechanical strength.

The gelatin structure was investigated as a function of gelatin concentration, ratios of $D_2O/H_2O$, temperature, pH, salt concentration and cation valence. The results can be summarized as follows:

1) With an increase of the gelatin concentration from a dilute solution to concentrated hydrogels, the clustering strength of gelatin chains increases and allows to form
aggregates of gelatin triple-helices resulting in gelation. Dehydration of the hydrogels to the dry state results in gelatin crystallization;

2) The inhomogeneous distribution and scattering length density of gelatin segments in the gelatin hydrogels results in two different contrast matching points of the 18 wt% sample for local structure and large clusters; while the diluted sample of 6wt% gelatin sample shows an overall minimum contrast at 28 v/v% of D$_2$O;

3) The structure of gelatin large clusters is stable below 60°C. The thermal transition of gelatin solutions from a gel to sol phase is accompanied by a triple-helix to random coil transition as well as by the decrease of the clustering strength. At lower temperature the hydrogel has more gelatin chains in the triple-helice-conformation and thus a higher gel strength;

4) Higher pH causes aggregation of large clusters of the large scale structure.

5) The monovalent ions Na$^+$ in the hydrogels do not change the gel structure. Divalent ions especially Ca$^{2+}$ in the hydrogels decrease the gel strength as well as the triple helix ratios in the dilute phase by a strong screening effect. The trivalent ions Fe$^{3+}$ show distinguished effects on the aggregation of large clusters, formation and growth of the gelatin triple-helices due to their junction role.

As a good medium for mineralization of inorganic ions, the choice of appropriate gelatin concentrations, temperature, pH will achieve high efficiency for ion transport (diffusion), optimal iron mineralization as well as high mechanical strength. Thus, we found and selected optimal concentrations of 6 wt% ≤ $c_{\text{gelatin}}$ ≤ 18 wt% for growing of magnetite nanoparticles in the hydrogels at room temperature.
Figure 3.24. Summary and schematic representation of the results of gelatin structure obtained from SANS/VSANS. $\phi$ is the gelatin volume fraction. The term $A/Q^n$ being the clustering strength and $\xi$ being the correlation length model obtained from the correlation length model fitting results (equation 3.4). The $R_g$ being the radius gyration obtained from the Beaucage equation fitting results (equation 3.1).
4 Superparamagnetic Magnetite Mineralization in Gelatin Hydrogels

4.1 Introduction

4.1.1 Superparamagnetic Magnetite

Magnetite (Fe₃O₄) is one of the three common naturally occurring iron oxides. It is the most magnetic of all the natural minerals on earth, which is usually found in magmatic, metamorphic and sedimentary rocks.¹⁵¹ Magnetite is also a biologically important magnetic iron oxide, it has received a lot of attention over the past several decades owing to the biocompatibility and high saturation magnetization. The magnetic behavior of a magnetite crystal depends upon the crystal's size and shape. The magnetite particles below a size threshold of 30 nm exhibit superparamagnetic behavior at room temperature. Such particles do not possess a stable magnetic order due to thermal fluctuation induced random flipping of the magnetic moment.¹⁵²,¹⁵³ Such particles exhibit a small net magnetic moment when an external magnetic field is applied. With the external magnetic field switched off, the overall net magnetic moment instantaneously falls back to zero. This feature makes them suitable for diverse applications such as contrast agents in magnetic resonance imaging (MRI),¹⁵⁴ targeted drug delivery¹⁵⁵ and biosensing¹⁵⁶ applications.

4.1.2 Coprecipitation Methods

The formation of superparamagnetic magnetite with controlled shapes and sizes can be achieved by numerous chemical methods, such as sol-gel syntheses¹⁵⁷, hydrothermal¹⁵⁸, syntheses microemulsions¹⁵⁹ and coprecipitation methods¹⁶⁰. Among the various methods for magnetite synthesis, the coprecipitation pathway is a fast and convenient way to synthesize magnetite nanoparticles from an aqueous iron (Fe²⁺ and Fe³⁺) solution.¹⁶¹ The process generally leads to small nanoparticles (< 20 nm) that show superparamagnetic properties. The chemical reaction of magnetite formation via a coprecipitation pathway can be written as
Fe$^{2+}$ + 2 Fe$^{3+}$ + 8 OH$^-\rightarrow$ Fe$_3$O$_4$ + 4 H$_2$O \hspace{1cm} (4.1).

Complete precipitation of magnetite should be expected at a pH between 8 and 14, with a stoichiometric ratio of 2:1 (Fe$^{3+}$/Fe$^{2+}$) at room temperature.\textsuperscript{151} The size and morphology of the magnetite nanoparticles can be tailored by adjusting pH, ionic strength, temperature and the Fe$^{3+}$/Fe$^{2+}$ ratio. Particles with size less than 20 nm can be thus obtained.\textsuperscript{151, 160, 161}

4.1.3 Bio- and Bioinspired Mineralization of Magnetite

In many cases biomineralization represents a sophisticated process forming a highly hierarchically ordered mineral structure controlled by the living organism\textsuperscript{24, 162, 163}. Biomineralization is executed under strict biological control of specially designed biomacromolecules, e.g. proteins\textsuperscript{164-166} and polysaccharides\textsuperscript{167}. An amazing example of biomineralization is the tooth formation of chitons consisting of a magnetite/protein-polysaccharide hybrid shell\textsuperscript{52}. The fully mineralized chiton tooth displays remarkable functional properties such as outstanding fracture toughness, wear resistance and has the highest reported hardness among known biominerals\textsuperscript{48, 168}. Proteins and a pre-formed polysaccharide fibrous hydrated network are buried within the teeth and provide a spatially confined template for the growth of a tooth's magnetite shell of defined geometry\textsuperscript{49}. It is quite evident that the interaction between the inorganic mineral and the organic matrix interface\textsuperscript{169} is essential for an understanding of the whole process, which may open new ways of new material design strategies and the generation of materials with improved chemical and physical properties. We thus attempted to explore, inspired by nature’s fabrication strategies, the use of gelatin molecules in the study of \textit{in-situ} mineralization of material with the mechanical properties of chiton tooth material, particularly shedding light on the mechanistic aspects of controlling the processes.

Gelatin is a soluble polypeptide derived from insoluble collagen through hydrolysis. The fibrous collagen molecule is formed by three individual polypeptide strands and has the conformation of a triple helix\textsuperscript{113}. Hydrolysis separates the collagen triple helix into three polypeptide strands composing to gelatin. Generally, gelatin hydrogels are attractive soft materials for biological applications\textsuperscript{120-122} owing to their hydrophilic insoluble properties with various functional groups (-NH$_2$, -COOH) and biocompatibility. Cavities inside the hydrogel could act as a protein scaffold, which, therefore, is an ideal natu-
ral protein assembly to control the spatial and structural order of the mineral. The last property makes the gelatin hydrogel a convenient platform to model a material via magnetite mineralization in order to achieve properties of teeth of Chiton.

4.1.4 The Aim of This Part

This part presents the design of a bio-inspired method for room temperature precipitation of superparamagnetic magnetite, which closely mimics the mineralization strategy taken by teeth of Chiton, using the gelatin matrices as the pre-formed network. We aim to explore the role of gelatin hydrogels on bio-inspired magnetite mineralization by using small (SANS) and very-small (VSANS) angle neutron scattering in conjunction with TEM/AFM/XRD measurements. For this purpose, we investigated mineralization of series of iron loaded gelatin precursors by systematically varying the iron content (c \( [\text{Fe}^{3+}/\text{Fe}^{2+}] \)) from the ratios of 5/2.5 to 200/100 in units of mM as well as the magnetite mineralization mechanism. The scattering contrast of the aqueous solution was adopted by the choice of D\(_2\)O content in order to determine separately the organic and inorganic components. We aimed to provide novel insights establishing a direct relation between the spacing confinement of the gelatin matrix and the mineralized particles producing such highly and optimized sophisticated materials properties.

The following presented data was performed in collaboration with different groups in a Priority program project of the DFG (SPP 1569 Generation of multifunctional inorganic materials by molecular bionics). Samples synthesis and parts of non-scattering characterization are closely collaborated with Maria Siglreitmeier (a Ph.D. student in AG Cölffen, Uni Konstanz). Parts of the presented results in this chapter were adapted from the following publications:


4.2 Experimental Section

4.2.1 Materials

Materials: The following commercially available chemicals were purchased and applied in the synthesis without further purification: FeCl₂·4H₂O (Sigma-Aldrich), FeCl₃·6H₂O (Sigma-Aldrich), NaOH (Sigma-Aldrich), D₂O (99.8% D) (ARMAR Chemicals), Gelatin Type B (∼225 Bloom, Sigma-Aldrich), 4-chloro-m-chresol (Fluka), Methanol (VWR). For the preparation of the reactant solutions double-distilled and deionized (Milli-Q) water was used. All iron including solutions were degassed with argon before use.

4.2.2 Synthesis of Gelatin Hydrogels

Different amounts of gelatin were allowed to swell in water for 24 hours at 5 (±1)°C. Homogeneous solutions were prepared by heating the gels for 2 hours at 45 °C. In each case, 10 mL of solution is filled in a glass dish (D = 11 mm) and allowed to form a hydrogel film with a thickness ~1 mm. To avoid decomposition by bacteria, a 5 wt% solution of 4-chloro-m-chresol in methanol was added (0.15 mL per 1 g of dry gelatin). All the samples for SANS-VSANS measurement were prepared in D₂O or mixture of D₂O/H₂O.

4.2.3 Magnetite Mineralization in Gel Matrices

In-situ mineralization of magnetite nanoparticles in a gelatin hydrogel was carried out via co-precipitation of FeCl₂ and FeCl₃. Each gelatin hydrogel film sample was introduced into 50 ml of aqueous FeCl₃ and FeCl₂ (concentration range from 5 mM FeCl₃/2.5 mM FeCl₂ to 200 mM FeCl₃/100 mM FeCl₂) and soaked for 96 hours at 5
Superparamagnetic Magnetite Mineralization in Gelatin Hydrogels

(±1)°C. The iron (III) and iron (II)-loaded gel film precursors were washed with water and placed in 0.1 M NaOH solution for 30 min. The resulting magnetite/gelatin composite films were washed 3 times with water respectively. The H₂O solvent was changed to D₂O or a mixture of D₂O and H₂O for all the SANS characterizations, respectively. The detailed experiment steps are schematically presented in Figure 4.1.

![Figure 4.1. Schematic representation of experimental steps. (1) Synthesis of gelatin hydrogels. (2) The gelatin hydrogel films. (3) Magnetite mineralization in the hydrogel matrices.](image)

### 4.2.4 SANS and VSANS Experiments

Small-Angle Neutron Scattering (SANS) and Very-Small Angle Neutron Scattering (VSANS) experiments were carried out at, respectively, the KWS1 and KWS3 diffractometers of JCNS outstation at the Heinz Maier-Leibnitz Zentrum (MLZ) in Garching, Germany. Four configurations were used at KWS1, namely the sample-to-detector (SD) distances of 1.3, 2, 8 and 20 m, the corresponding collimation length of 8 and 20 m, and a wavelength of 7 Å (Δλ/λ = 10%). These settings allowed covering a Q-range from 0.002 to 0.35 Å⁻¹. The scattering vector Q is defined as Q = 4π/λsinθ with the scattering angle 2θ and the wavelength λ. A two-dimensional position sensitive detector was used to detect neutrons scattered from sample solutions. Sample films characterized by SANS and VSANS were hydrated in D₂O or mixture of D₂O/H₂O and placed in a sample cell between two quartz windows (sandwich quartz cell, see Appendix B) with path length of 1 mm. Plexiglas was used as a secondary standard to calibrate the scattering intensity in absolute units at KWS1 and direct beam method at KWS3. The data correc-
tion and calibration were performed using the software QtiKWS\textsuperscript{96}. Fitting the SANS data was done using software modules provided by JCNS QtiKWS\textsuperscript{96} and NIST Igor\textsuperscript{127} analysis packages. In order to cover the broader length scale of the network structure, very-small angle neutron scattering experiments were carried out at the KWS3 diffractometer using the parabolic mirror as an optical element, and covering the smaller Q range from 0.0001 to 0.002 Å\textsuperscript{-1}.\textsuperscript{92}

4.2.5 SAXS Experiments
SAXS experiments were carried out at a HECUS S3-Micro and GALAXI\textsuperscript{97} small-angle X-ray scattering instrument. The HECUS S3-Micro instrument uses Cu K\textalpha radiation (0.154 nm) produced in a sealed tube. The GALAXI instrument uses a Metaljet source (λ=1.34 Å). Gel samples were placed in Hilgenberg quartz capillaries with an outside diameter of 1 mm and wall thickness of 0.01 mm. The scattered intensity was corrected with the transmission of the samples calculated considering the absorption of the sample and that of the capillary. The dry gel samples were cut to a thin film with a thickness 1mm and measured directly. The scattered X-rays are detected with a two-dimensional multiwire area detector and afterwards converted to one-dimensional scattering by radial averaging and represented as a function of the momentum transfer vector Q similar to the SANS experiments.

4.2.6 Atomic Force Microscopy (AFM)
Non-contact mode atomic force microscopy (AFM) using an Agilent 5500 microscope was employed to study the 3D structure of the magnetite-gelatin dry films.

4.2.7 Transmission Electron Microscopy (TEM)
For TEM analysis, a Zeiss Libra 120 operating at 120 keV and a JEOL JEM-2200FS operating at 100 keV were used, respectively. For material characterization, a drop of a diluted dispersion of nanoparticles extracted from the hydrogel was placed on a formvar carbon coated copper grid and left to dry on a filter paper for measurements.
4.2.8 Powder X-ray diffraction (XRD)

XRD patterns were recorded on an image plate camera (Model G670, Huber, Cu Kα1 radiation, \( \lambda = 1.54 \) Å, quartz monochromator, Guinier camera). Diffraction data were collected between 3° and 100°.

4.2.9 Superconducting Quantum Interference Device (SQUID)

Magnetization measurements were carried out by using a quantum design SQUID 5 T magnetic property measurement system (MPMS). For measurements, dried gel samples were introduced into gelatin capsules and magnetization loop measurements at 2 K and 293 K were performed. In addition zero-field-cooled and field-cooled curves were obtained by applying 0.01 T and heating or cooling the sample. The measurements were done by Maria Siglreitmeier in Uni-Konstanz.

4.3 Theoretical Part

4.3.1 Model for Small-Angle-Scattering

The SANS and VSANS diffractometers deliver scattering data from very small Q of the order of \( 10^{-4} \) up to 0.35 Å\(^{-1} \), covering length scales from 20 Å up to 6 µm. The Q is scattering vector where \( Q = 4\pi \sin \theta / \lambda \) with \( 2\theta \) of the scattering angle and \( \lambda \) neutron wavelength. Some expression and models for the hybrid materials as following:

**The Beaucage Expression**

The Beaucage empirical expression is able to reasonably approximate the scattering from many different types of particles, including fractal clusters, random coils (Debye equation), ellipsoidal particles, etc.\(^{103} \) Beaucage expression is given according to:

\[
\frac{d\Sigma}{d\Omega}(Q) = \frac{d\Sigma}{d\Omega}(0) \exp\left(-u^2 / 3\right) + P_\alpha [(\text{erf}(u / \sqrt{6}))^3 / Q]^{\alpha} (4.2)
\]

representing a combination of Guinier’s and Porod’s laws describing the scattering at low and large Q, respectively. More quantitatively both approximations are valid for the parameter \( u = R_g Q \) smaller or larger than 1, \( u \) representing the product of radius of gyration \( R_g \) and scattering vector Q. Guinier’s law has the shape of a Gaussian function whereas for Q larger than 1/R\(_g\) (\( u > 1 \)) a power law according to \( d\Sigma / d\Omega(Q) = P_\alpha Q^{-\alpha} \) is
often observed. Those empirical expressions can also be used in a multi-level model for the sample have a multi-scale structure.

In this chapter, the scattering curves from the inorganic minerals as well as the gelatin triple-helices in the organic matrix were fitted by Beaucage expression to generate the size and dimensions of a sample.

**The Correlation Length Model**

The correlation length model developed by B. Hammouda\textsuperscript{104} is made of two terms. The first term describes Porod scattering from clusters (exponent = n) at small Q and the second term is a Lorentzian function describing scattering from polymer chains (exponent = m) at larger Q. The second term \( C/[1+(Q\xi)^m] \) characterizes the polymer/solvent interactions and therefore the thermodynamics. The two multiplicative factors A and C, the incoherent background BKG and the two exponents n and m are used as fitting parameters.\textsuperscript{79, 104}

\[
\frac{d\Sigma}{d\Omega} (Q) = \frac{A}{Q^n} + \frac{C}{1+(Q\xi)^m} + BKG
\] (4.3)

In the case of the polymer clusters, the first term \( A/Q^n \) becomes a key parameter to evaluate the clustering strength. In this chapter, the composites in D\textsubscript{2}O corresponding to the organic matrix structures are fitted with the correlation length model to generate the correlation length and to evaluate the clustering strength.

**4.3.2 Molecular Simulation**

Our collaboration group from Erlangen (AG D.Zahn) performed molecular simulation studies of Fe\textsuperscript{2+}/Fe\textsuperscript{3+} and hydroxide ion association to a triple-helical (Gly-Hyp-Pro)\textsubscript{n} peptide.\textsuperscript{170}

A series of FeIII(OH)\textsubscript{x}(OH\textsubscript{2})\textsubscript{4-x} and FeII(OH)\textsubscript{y}(OH\textsubscript{2})\textsubscript{8-y} clusters were pre-modeled from ab-initio calculations in vacuum. For all clusters, the high-spin constellation was identified as preferred by several electron volts. Imposing overall charge neutrality (i.e. \( x+y=3+2 \)) we found the neutral FeIII(OH)\textsubscript{3} \cdot H\textsubscript{2}O and FeII(OH)\textsubscript{2} \cdot 6 H\textsubscript{2}O clusters as energetically preferred. Docking to collagen was modeled in aqueous solution using empirical force-fields. Investigation of biologically-designed metal-specific chelators for potential metal recovery and waste remediation applications, and the Kawska-Zahn
docking procedure were used as described previously\textsuperscript{171}. Along this line, ion clusters are initially docked to collagen in absence of water. Such putative association complexes are then immersed in aqueous solution (periodic simulation cell comprising more than 15,000 water molecules) and subjected to relaxation from 100 ps molecular dynamic runs at room temperature and ambient pressure. To account for the manifold possible arrangements intrinsic to the systems complexity, a series of 100 independent docking runs were performed for each ionic species. The resulting structures were then classified in terms of hydrogen bonds and O·∙Fe distances to discriminate the representative configurations of the FeIII(OH)$_3$·O (collagen) and FeII(OH)$_2$·O (collagen or H$_2$O) coordination constellations.

4.4 Results and Discussion

4.4.1 The Iron-loaded Gelatin Precursors

To form magnetite-gelatin hydrogel composites by co-precipitation of Fe$^{3+}$ and Fe$^{2+}$ ions, gelatin hydrogel films were first soaked 24h up to 96h at \textasciitilde{}5\degree C in an aqueous iron ion solution. Combined SANS and VSANS data are shown in Figure 4.2 (a) can be used to depict structure changes in the iron loaded hydrogel networks as the magnetite mineralization precursor. In order to investigate the different iron ion effects on the gelatin structure, we have measured the Fe$^{3+}$-Gel (Gelatin gel soaked with 0.3 M FeCl$_3$ solutions), Fe$^{2+}$-Gel (Gelatin gel soaked with 0.3 M FeCl$_2$ solutions) and the mixture of Fe$^{2+}$- Fe$^{3+}$-Gel with a molar ratio of ferrous to ferric ions of 1:2 by SANS/VSANS. We observe in Figure 4.2 (a) that each of the curves is slightly varying, but essentially constant for the iron loaded gel precursors with Fe$^{3+}$ ions (Gel-Fe$^{3+}$ and Gel-Fe$^{2+}$-Fe$^{3+}$) or without Fe$^{3+}$ ions (Gel-Fe$^{2+}$ and Gelatin-Gel 12 wt\% in D$_2$O).

In the lower Q regime below 0.01 Å$^{-1}$, the scattering of the gel precursors containing Fe$^{3+}$ ions shows fractal structure (Figure 4.3). The power law exponent is around 2.7 for samples containing Fe$^{3+}$ ions indicating a mass fractal and infinite-size gel structure while the samples without Fe$^{3+}$ ions (e.g. Gel-Fe$^{2+}$ and Gelatin-Gel 12 wt\% in D$_2$O) plateaus to a nearly constant value indicating the appearance of finite size structure within the gel. Such variation might arise from changes in the large-scale structure of the hydrogels, particularly the aggregation of the larger clusters as the Fe$^{3+}$ ions are loaded. This result reveals that Fe$^{3+}$ ions provide cross-linkers between gelatin side-
chain-residues which make the larger clusters aggregate. In contrast, SANS/VSANS results show that Fe$^{2+}$ ion loaded gelatin exhibits similar structure with the iron-free gelatin samples which shows the poor interaction introduced crosslinks by Fe$^{2+}$ ions.

**Figure 4.2.** (a) SANS-VSANS scattered intensity versus scattering vector Q for iron-loaded gelatin in D$_2$O (T = 20 ±2°C). In a Q range above 0.01 Å$^{-1}$, the solid line represents the best fit by correlation length model of equation 4.3. In the Q range below 0.01 Å$^{-1}$, the scattering curves were fitted by Beaucage expression of equation 4.2(Fe$^{3+}$ ions consisting gels) and power law (Fe$^{3+}$ ions free samples). Parameters from SANS-VSANS pattern of iron loaded gelatin samples: (b) the scattering intensity at Q =0.04 Å$^{-1}$, (c) the high-Q feature correlation length $\xi$, (d) the iron loaded hydrogel average cage size $R_m$ (e) the high Q power law exponent $\beta$ (0.04 < Q < 0.4 Å$^{-1}$). The parameters from (b), (d) and (e) obtained from the SANS-VSANS results in Fig. 4.2 (a) while $\xi$ are obtained from the fitting results from equation 4.3.

In the Q regime above 0.01 Å$^{-1}$, the high-Q scattering is distinctively different in the iron loaded gels (with or without of Fe$^{3+}$). The helix structure is more compact and therefore characterized by a higher scattering intensity. Thus data (Figure 4.2a, and the enlarged section) show an abrupt decrease in the high-Q intensity for the gelatin triple-helix-rich phase but a gradual decrease for the gelatin random coils.
Figure 4.2b, c, d and Table 4.1 summarize some of the important parameters obtained by the SANS data and the fitting results.

Figure 4.2b shows the variation of the scattering intensity at $Q = 0.04 \text{ Å}^{-1}$ for iron-loaded gels. The intensity drop from 0.35 $\text{ Å}^{-1}$ to 0.65 $\text{ Å}^{-1}$ between $\text{Fe}^{3+}$-consisting samples and $\text{Fe}^{3+}$-free samples characterizes the coil to triple-helix transition. Moreover, the solvation intensity $C$ by fitting the data with equation 4.3 (Table 4.1) decreased significantly when $\text{Fe}^{3+}$ ions were loaded in the gel. Figure 4.2c summarizes the variation of the correlation length ($\xi$ in equation 4.3) of the iron-loaded samples. This correlation length represents a weighted average inter-distance between the hydrogen/deuterium-containing groups. The value is 40.5 ($\pm 0.8$) Å in the iron-free sample (Gel 12 wt% in $\text{D}_2\text{O}$) and decreases to 28.9 ($\pm 0.9$) Å in the $\text{Fe}^{3+}$-consisting samples (Gel-$\text{Fe}^{3+}$ in $\text{D}_2\text{O}$). In the triple helix conformation, the inter-distance of the gelatin chains is shorter than in the random coil conformation. Thus, the decrease in $\xi$ is partly due to the increase the triple-helix configuration in the hydrogels by the addition of $\text{Fe}^{3+}$ ions. Figure 4.2 (d) represents the variation of the average 3D-cage size ($R_m$) which is estimated from crossover point ($Q_c$) of the intermediate scattering curve. This value is seen to increase from 279 ($\pm 70$) Å in the $\text{Fe}^{3+}$-free samples (Gel 12 wt% in $\text{D}_2\text{O}$) to 356 ($\pm 40$) Å in the $\text{Fe}^{3+}$ containing sample (Gel-$\text{Fe}^{3+}$ in $\text{D}_2\text{O}$). Alternatively, such variation might arise from increases in triple-helix ratios of the gel network, increasing the length and also the radius of the gelatin triple-helices (increasing of $R_g$) as the $\text{Fe}^{3+}$ ions are loaded into the Gel. Finally, in Figure 4.2 (e), the high-$Q$ Power law exponent $\beta$ is seen to vary between values around 2 in the $\text{Fe}^{3+}$-consisting sample to values close to 1.4 in the $\text{Fe}^{3+}$-free samples. It is noted that the rod-like nature of gelatin triple helix bundles (Power law exponent $\sim 1$) have not been seen due to the clustering signal overwhelming the low-$Q$ scattering.

Figure 4.3 is schematic representing the structure changes with loading of iron ions. The $\text{Fe}^{3+}$ ions loaded hydrogels show significantly increasing scattering intensity in combination with the dramatically increasing of $R_g$, clustering strength, and the gel 3D-cage size. The changes indicate the formation of more triple-helix, growing the helices length, and aggregation of the triple-helices (growing the rod-like diameter). Thus, the $\text{Fe}^{3+}$ ions show a strong crosslinking effect on the gelatin network which are considered as junctions in between the gelatin inter-chains.
Figure 4.3. Schematic representation of gelatin hydrogel structure changes with Fe$^{3+}$ and Fe$^{2+}$ loading.

Table 4.1. Parameters of iron ions-loaded Gel in D$_2$O obtained from the SANS-VSANS data and fitting results.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Gel 12wt% / D$_2$O</th>
<th>Gel-Fe$^{2+}$</th>
<th>Gel-Fe$^{3+}$</th>
<th>Gel-Fe$^{3+}$-Fe$^{2+}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>dΣ/dΩ(Q=0.04 Å$^{-1}$)/cm$^{-1}$</td>
<td>0.380 ±0.006</td>
<td>0.329 ±0.005</td>
<td>0.708 ±0.008</td>
<td>0.636 ±0.008</td>
</tr>
<tr>
<td>α</td>
<td>2.9</td>
<td>2.7</td>
<td>2.7</td>
<td>2.7</td>
</tr>
<tr>
<td>Qc / Å$^{-1}$</td>
<td>±0.0045</td>
<td>± 0.001</td>
<td>±0.0016</td>
<td>±0.001</td>
</tr>
<tr>
<td>R_m / Å</td>
<td>279 ±70</td>
<td>314 ±17</td>
<td>356 ±42</td>
<td>369 ±55</td>
</tr>
<tr>
<td>R_g / Å (Regime II)</td>
<td>46.2 ±0.1</td>
<td>49.4 ±0.3</td>
<td>132 ±1</td>
<td>120 ±1</td>
</tr>
<tr>
<td>C</td>
<td>1.06 ±0.02</td>
<td>0.90 ±0.6</td>
<td>0.011 ±0.001</td>
<td>0.0086 ±0.0004</td>
</tr>
<tr>
<td>m</td>
<td>1.63 ±0.007</td>
<td>1.31 ±0.06</td>
<td>3.27 ±0.12</td>
<td>2.98 ±0.09</td>
</tr>
<tr>
<td>A/Qn (Q=0.02 Å$^{-1}$)</td>
<td>0.58</td>
<td>0.79</td>
<td>0.73</td>
<td>0.78</td>
</tr>
<tr>
<td>ξ / Å</td>
<td>40.5 ±0.8</td>
<td>43.7 ±4</td>
<td>28.9 ±0.9</td>
<td>27.8 ±0.8</td>
</tr>
<tr>
<td>β</td>
<td>1.2</td>
<td>1.3</td>
<td>1.7</td>
<td>1.7</td>
</tr>
<tr>
<td>BKG</td>
<td>0.074</td>
<td>0.082</td>
<td>0.121</td>
<td>0.126</td>
</tr>
</tbody>
</table>

From the above discussion, the SANS-VSANS results show that Fe$^{3+}$ have not only an effect on the large-scale structure by cross-linking the gelatin side chain/aggregation of the larger clusters but also assist with formation of a higher ratio of gelatin triple-helices as well as growth of the triple-helices by acting as junction roles. The feature of the Fe$^{2+}$
ion loaded gel apparent from the scattering data is very close the pure gelatin gel indicating that the Fe²⁺ ions have poorly bridging effect for changing the network structure. Thus in the mixture of Fe²⁺/Fe³⁺ loaded gel precursors (with lower iron ion load hydrogels), the two iron ions are playing different roles in the gel matrix. The Fe²⁺ ions act as free ions in the gel matrix while Fe³⁺ ions are bound with the amino residues and COO⁻. The Fe³⁺ ions mediate formation of additional triple helices and further increase (percolation) the dense phase to such a size of above 5 µm. The interaction between the hydrogels and the two ions may influence on the magnetite mineralization.

**4.4.2 Magnetite Mineralization in the Iron-Loaded Gelatin Hydrogels**

The magnetite was formed inside the gelatin network after immersing the iron loaded gel precursor (gelatin soaked in a 200 mM Fe³⁺ and 100 mM Fe²⁺ contained solutions) into a NaOH (0.1 mol L⁻¹, pH ≈ 11) solution. It is clear that gelatin hydrogel matrices are excellent for enhancing the aqueous stability of magnetite nanoparticles and have the ability to control growth and influence the crystal size by the spatially confined matrix structure.¹⁷⁰

**TEM and AFM Results (real space images on the materials)**

Figure 4.4 (a) shows TEM result of the composites, where a homogenous distribution of the magnetite nanoparticles with 90 (±30) Å in diameter with cubic like shape (the inset picture) coexist with polymer chain-like matrix. The nanoparticles do not show any uncontrolled aggregation which might be due to stabilization by gelatin. The arrangement of the crystallites along the gelatin triple helices can be attributed to the mineral precursors as the nuclear center which attach along the gelatin matrix as shown in the insets of Figure 4.4 (a). The TEM image of Figure 4.4 (b) shows that in the absence of gelatin matrices, magnetite has particles with a higher average size and clearly aggregates compared to the magnetite mineralization in the presence of gelatin matrix.
Figure 4.4. TEM images of magnetite mineralization (a) within and (b) without gelatin hydrogels. The concentration of the hydrogels is 18 wt% of gelatin.

Figure 4.5. AFM images of the dry composite films. (A) and (C) are the phase contrast images. (B) and (D) are the amplitude images.
Figure 4.5 presents AFM images of magnetite-gelatin dry composites. Figure 4.5 (A) is an AFM phase contrast image which shows a large area of homogenous distribution of the magnetite nanoparticles (the light dots) with some large gelatin domain (in blue color, the large gelatin clusters). Figure 4.5 (B) is the AFM amplitude image of sample morphology which shows clearly a fractal-like 3D gelatin (gold color) network with nanoparticles (the bright dots) inside. Figure 4.5 (C) shows a phase contrast image in which the bright dot-like particles indicate a ~90 Å in diameter of the colloidally stable magnetic nanoparticles inside the fractal gel structure. The dry fractal gels show a much thicker matrix wall of tens of Å than the gelatin hydrogen of 10 Å (Gelatin 18 wt %). The result suggests an assembly of gelatin triple-helices to a fiber during the drying process. The average cage size of the dry matrix was estimated from AFM data to be ~ 500 Å which is slightly larger than the value of the hydrogel. Furthermore, Figure 4.5 (D) presents an image by using AFM amplitude with a higher tapping force. By using a strong force on the tip, some nanoparticle imprints were found on the image. The result shows that the particles are following a distribution attached to the gelatin matrix.

**The Magnetic Measurements**

The magnetic properties of these composite materials were characterized by using a SQUID magnetometer. Figure 4.6 (a) shows the magnetization (M) of a dried sample (made from 10 wt% gelatin) as a function of the applied field (H) at 293 K and 2 K. Temperature at T = 2 K the magnetization curve shows typical ferrimagnetic hysteresis due to magnetic anisotropy. At 293 K the hysteresis disappeared indicating a typical superparamagnetic properties with the nanoparticle size smaller than 30 nm. The field-cooled (FC) and zero-field-cooled (ZFC) magnetizations of the magnetite-gelatin composites were also measured (Figure 4.6b). The maximum of the ZFC curve corresponds to the blocking temperature (TB). The results show a blocking temperature of ~120 K which also confirms the superparamagnetic behavior of the nanoparticles. Thus the observations indicate that the magnetite nanoparticles show typical superparamagnetic properties.
Figure 4.6. Magnetic properties of the synthesized hybrid materials.\textsuperscript{170} a) Magnetization curves of a dried ferrogel at 2 K and 293 K. b) ZFC-FC curves as a function of temperature.

The mineral phase structure, XRD

The mineral phase structure in the composites was investigated by XRD spectra in Figure 4.7 with the dry gelatin films for comparison. The XRD spectrum of pure dry gelatin displays three main peaks, at $2\theta = 7.6^\circ$, $\sim 20^\circ$, and $31.9^\circ$ depicting the diameter of the triple helix (repeat distance of 11.7 Å), the amorphous halo, and the amino acid contacts along the axis of single helices (repeat distance of 2.8 Å), respectively.\textsuperscript{138} In contrast, the intensity of gelatin peaks indicating single or triple helical regions ($2\theta = 31.9^\circ$ or $7.6^\circ$) were significantly decreased in the composites reflecting poorly crystalli-
Superparamagnetic Magnetite Mineralization in Gelatin Hydrogels

It was proved that the mineralization in the gelatin matrix enabled the suppression of gelatin chain helicity, which is crucial for the mechanic properties of a gelatin network with defined structure-property relationships. In the XRD result of the composites, XRD peaks at 35.5°, 57°, and 62.5° match the typical diffraction patterns observed for magnetite (JCPDS Card No. 19-0629). The average diameter of the magnetite nanocrystals was estimated from the Scherrer’s equation by which the nanocrystal size calculated by Scherrer’s equation is around 90 Å. The size is in good agreement with the particles size measured by TEM indicating that the nanoparticles are made of single crystal domain.

![XRD peaks of dry gelatin and gelatin-magnetite composites](image)

**Figure 4.7.** XRD of the dry gelatin and gelatin-magnetite composites.

**Contrast variation studies of the gelatin-magnetite composites**

In order to further clarify the structure of the gelatin-nanoparticle hybrid material, we performed SANS contrast variation experiments, which allowed to independently explore the inorganic nanoparticle structure as well as the gelatin gel network. Contrast variation SANS experiments became a standard method as pointed out and is applied also in cognate disciplines such as biomineralization. Figure 4.8 displays two SANS scattering patterns of composites (prepared from gelatin 18 wt%) dissolved in pure D₂O and in an aqueous mixture of 28 vol% D₂O matching the scattering of magnetite and gelatin, respectively.
In D$_2$O, when gelatin is the only visible part, the scattering at small $Q$ ($Q < 0.001$ Å$^{-1}$) delivers a fractal large cluster aggregation as found for Fe$^{3+}$ loaded gelatin (in Figures 4.2) and gelatin in base conditions (in Figures 3.21). The large $Q$-regime shows similar scattering comparing with pure gelatin suggesting a cage-like network composed of triple-helix and random coils.

**Figure 4.8.** SANS-VSANS scattering pattern of the gelatin-magnetite composites (sample prepared from 18 wt% gelatin hydrogels) in pure D$_2$O and a mixed D$_2$O/H$_2$O solvent of 28 vol% D$_2$O and 72 vol% H$_2$O. The blue solid lines represent the fitting of the Beaucage expression (equation 4.2) at high $Q$ and power law in the low $Q$. The red solid lines represent the fitting of the correlation length model (equation 4.3) at high $Q$ and power law in the low $Q$. In a box of the inset picture there is a shape reconstruction result from the scattering of the sample by using Dammif. The yellow open circles in Figure 4.8 represent the scattering of the composite in a 28 vol% D$_2$O aqueous solution, which matches the gelatin scattering and visualizes the magnetite nanoparticles. There is weakly enhanced scattering as high as the scattering of composites in D$_2$O in the small $Q$-range, which might cause from a scattering of necklace-like nanoparticle ‘chains’. The scattering in the intermediate $Q$-range is caused by individual nanoparticles of $R_g = 45.4 \pm 0.1$ Å showing a $Q^{-3}$ power law at intermediate $Q$ which might indicate a rough surface. The spherical diameter $D$ of the magnetite
particles can be estimated as $D \sim 117 \pm 0.3 \text{ Å} \ (D = 2\sqrt[3]{5/3}R_g)^{70}$. This value is in agreement with the result obtained from TEM (diameter 90 ±30 Å) and AFM, but of smaller size than the cage of the hydrogels (~260 Å). However, the SANS result is slightly larger than the TEM/AFM which may be caused by the rough surface of the particles. Figure 4.3 inset in a box are the reconstruction results from the SANS data in 28 vol% D$_2$O by a rapid ab initio shape construction software DAMMINF$^{134}$. For the shape reconstructed object, it seems that is not a spherical particle but some shape in between cubic and elliptical particles, which also influence an accurate calculation of the diameter. A particle size smaller than the cage size means that magnetite formation does not destroy the cage-like gel structure. Moreover, the results suggest that the gel matrix determines the size of the magnetite as it prevents the nanoparticles from further growth as well as from aggregation.

**The organic matrix structure**

![Figure 4.9. SANS-VSANS scattering pattern of the gelatin-magnetite composites in pure D$_2$O, iron-loaded gel and pure gelatin 18 wt% in D$_2$O. The solid lines represent the fitting by correlation length](image-url)
model of equation 4.3 ($Q > 0.01 \, \text{Å}^{-1}$), Beaucage expression (equation 4.2) of pure gelatin 18 wt% in D$_2$O ($Q < 0.01 \, \text{Å}^{-1}$) and power law of gelatin-magnetite composites and iron-load gel in D$_2$O ($Q < 0.01 \, \text{Å}^{-1}$).

Figure 4.9 displays SANS/VSANS scattering patterns of magnetite/gelatin gels dissolved in pure D$_2$O and matching the scattering of magnetite. SANS/VSANS for pure gelatin gels in D$_2$O (Gelatin 18 wt%) and Fe$^{3+}$/Fe$^{2+}$ loaded gel precursors (In Gelatin 18 wt% in D$_2$O, with 0.2 M Fe$^{3+}$ and 0.1 M Fe$^{3+}$) are for comparison. In D$_2$O, when gelatin is the only visible part, the scattering at high Q ($Q > 0.02 \, \text{Å}^{-1}$) delivers a $Q^{1}$ rod-like power law scattering which is similar to the pure gelatin of 18 wt%. However, the scattering intensity is only $\sim$25% comparing with the pure gelatin 18 wt% in D$_2$O which is caused by the swelling effect. Thus, a lower gelatin concentration hydrogel (12 wt %) was compared with the composites (in D2O) on the right side of Figure 4.9. In the low Q regime, the scattering is a significant variation to the pure Gelatin. In the latter case in the very low-Q regime, the scattering curve is trend to a flat Guinier scattering which yields a very large radius of gyration of $R_g = 1590 \pm 30 \, \text{Å}$ of the large clusters. In contrast, in the low Q regime magnetite/gelatin hydrogels in D$_2$O show a $Q^{2.5}$ power law scattering corresponding to a mass fractal structure (branched structure). The scattering is very similar to the Fe$^{3+}$ loaded hydrogel precursors (show the right side of Figure 4.9). Thereby, the gelatin structure is an irreversible return to the original structure during the magnetite mineralization due to the irreversible aggregation of large clusters. However, the gel structure could return to the iron loaded gel precursor structure for both small scale and large scale.

In order to reach a high loading magnetite, usually the mineralization experiments are repeated for several times in order to increase the mineral content. It is interesting for the structure of the gelatin hydrogels, whether the structures change or not in many reaction cycles. Figure 4.10 compares the results when this mineralization experiment repeat in zero (the original hydrogel of Gelatin 12 wt% in D$_2$O), one and two cycles. Results show that the samples in a one reaction cycle and two reaction cycles have similar scattering curve meaning no significant change in their structures. There is the only difference between the sample after the mineralization and the original gel that is the aggregation of large gelatin clusters evident in the very low Q regime.
Superparamagnetic Magnetite Mineralization in Gelatin Hydrogels

4.4.3 The Iron Concentration Influence on the Mineralization

Figure 3.16 reveals the SANS contrast measurement of the gelatin 6 wt% in an aqueous mixture of H$_2$O and D$_2$O. The gelatin sample was dissolved in a 28 vol% D$_2$O solvent that matches with the gelatin contribution so that the signal is mostly arising from the inorganic nanoparticles. Figure 4.11 displays SANS scattering patterns of an iron mineralized hydrogel as a function of initially soaked iron concentration dissolved in an aqueous mixture of 28 vol% D$_2$O matching the scattering of gelatin and visualizes the magnetite nanoparticles. In the high Q regime (0.01 < Q < 0.15 Å$^{-1}$), the overall scattering intensity increased with the increase of the initial iron concentration throughout the measured Q range. The scattering of Fe200 (samples soaked in iron solutions with Fe$^{3+}$ 200 mM and Fe$^{2+}$ 100 mM, respectively) and Fe100 samples reveal a shoulder region indicative of a particles morphological feature with a radius of gyration, $R_g$, of 45.4 ±0.1 Å and 51.5 ± 0.3 Å for Fe200 and Fe100. The diameter D of the magnetite particles can be estimated as $D = 117$ (±0.3) Å and $D = 133$ (±0.9) Å ($D = 2\sqrt{5/3}R_g$ for spherical particles) for Fe200 and Fe100. This value is slightly larger than obtained from TEM (D = 90 (±30) Å) may due to the non-perfect spherical shape for diameter calculation from $R_g$ and rough parti-
cle surface. By contrast, the Fe10 and Fe20 samples do not show particle like scattering in the full Q range.

Figure 4.11. SANS scattered intensity versus scattering vector Q for magnetite formation in gelatin in a mixture D$_2$O/H$_2$O solvent of 28 vol.-% D$_2$O and 72 vol.-% H$_2$O (T = 20 ±2°C). The composites were synthesised in the 18 wt% gelatin hydrogels. The solid line represents fit of the correlation length model of equation 4.3 (Fe10, Fe20, Fe50), Beaucage equation (High Q feature of Fe100, Fe200) and Power law (Low Q feature of Fe100 and Fe200, the scattering intensity is following a Q$^{-2.5}$ scattering).

Kratky plots of dΣ/dΩ(Q)* Q$^2$ vs Q (Figure 4.12) exhibit a single distinct peak in the SANS region (0.01 < Q < 0.15 Å$^{-1}$) for samples of Fe200 and Fe100, which is typically indicative of globular structures. The peak at low Q followed by an elevated baseline at high Q indicates that the particles attach to a chain (see the Figure 4.11 inset picture). For samples of Fe10 and Fe20, Kratky plots show a chain like conformation. Thus, the SANS results reveal that a minimum initial soaked iron concentration of c[Fe$^{3+}$/Fe$^{2+}$] ≈50 mM/25 mM for magnetite nanoparticle formation.
Figure 4.12. Kratky plots of the SANS pattern from Figure 4.10 as a function of iron loading concentration.

The low Q regime ($Q < 0.01 \, \text{Å}^{-1}$), the $Q^{-2.5}$ power law (Figure 4.11) for the Fe200 and Fe100 samples indicates a mass fractal structure. The exponent $\alpha$ is slightly smaller than the value of pure gelatin gels of ~3 for Gelatin 18 wt% in D$_2$O in the lower Q range. The results imply that the nanometric inorganic objects organized to form bigger fractal objects along the gelatin matrix by a weak bridge effect. In contrast to Fe200 and Fe100, the power law exponents of 1.3 and 1.4 (Figure 4.11 and Table 4.2) for samples Fe10 and Fe20 indicate an object associated with the gelatin matrix to enhance the contrast of gelatin matrix thus visualization the matrix contribution. Thus, for the very low initial soaked iron concentration (e.g. $c[Fe^{3+}/Fe^{2+}] \approx 10 \, \text{mM}/\, 5 \, \text{mM}$), no particles form but some iron oxide clusters appeared.

Figure 4.13 is a schematic representation of the mineral shape dimension as a function of loading iron concentration. At lower concentration of $Fe^{3+}/Fe^{2+}$ iron pairs, e.g. Fe 10 and Fe 20, $Fe^{3+}$ ions act as bridging ions for formation and growth and aggregation of triple-helices. Thus increasing the pH to 11 there is no magnetite formation, but some other iron II produced precipitates attach to the gelatin networks. Further increasing the concentration of $Fe^{3+}/Fe^{2+}$ iron pairs, free $Fe^{3+}$ appeared and co-precipitates with $Fe^{2+}$.
products. Magnetite particles form in the samples of Fe100 and Fe200. The shape of the particles is transformed from elongate particles to approximate spherical particles indicating less influence from the gelatin chain.

Table 4.2. Parameters of Magnetite-Gel in D2O/H2O (D2O Vol 28%).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Fe10</th>
<th>Fe20</th>
<th>Fe50</th>
<th>Fe100</th>
<th>Fe200</th>
</tr>
</thead>
<tbody>
<tr>
<td>α</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>2.54</td>
<td>2.56</td>
</tr>
<tr>
<td>β</td>
<td>1.3</td>
<td>1.4</td>
<td>1.5</td>
<td>3.0</td>
<td>3.0</td>
</tr>
<tr>
<td>Rg/Å</td>
<td>-</td>
<td>-</td>
<td>172±5</td>
<td>51.5±0.3</td>
<td>45.4±0.1</td>
</tr>
<tr>
<td>ξ/Å</td>
<td>16.5±4.1</td>
<td>25.2±4.4</td>
<td>23.7±2.1</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

* The power law exponents α were obtained from Q < 0.01 Å, β obtained from Q > 0.01 Å.

Figure 4.13. Schematic representation of the shape dimension as a function of loading iron concentration.

In order to investigate the effect of mineralization on the gelatin chain helicity and mineral phase as a function of initial iron soaked concentration, XRD spectra of magnetite-gelatin composites films were compared with the dry gelatin films. A typical XRD spectrum of pure dry gelatin displays three main peaks, at 2θ = 7.6°, ~20°, and 31.9°. These peaks (yellow circle marked) depict the diameter of the triple helix (repeat distance of 11.7 Å), the amorphous halo, and the amino acid contacts along the axis of single helices (repeat distance of 2.8 Å), respectively. In contrast, the intensity of gelatin peaks indicating single or triple-helical regions (2θ = 31.9° or 7.6°) were decreased in the higher iron loaded dry gels (e.g. Fe200). However, the triple-helical peaks still appear in the hybrid gels. Therefore, it was proven that the mineralization in the gelatin matrix enabled the suppression of gelatin chain helicity, which is crucial for the mechanic
properties of a gelatin network with defined structure-property relationships. The dashed line marked XRD peaks at 35.5°, 57°, and 62.5° match the typical diffraction patterns observed for magnetite (JCPDS Card No. 19-0629). In Figure 4.14, the magnetite phase appeared when the initial iron soaked concentration is higher than \( c[Fe^{3+}/Fe^{2+}] \approx 200 \text{ mM}/10 \text{ mM} \) for Fe50, Fe100, Fe200. The results are in agreement with the SANS conclusions in which the particular scattering appeared only for the sample above the threshold value. Moreover, there is another peak appearing in Fe20 and vanishing in Fe50 at \( \sim 34.5° \). The structure may come from the \( Fe^{2+} \) precipitation products when \( Fe^{3+} \) ions are bound with gelatin. For all the samples, the average diameter of the magnetite nanocrystals was estimated from the Scherrer equation \( 174 \). In the case of Fe200, the nanocrystal size calculated by Scherrer’s equation is around 90 Å. The size is in good agreement with the particle size measured by TEM indicating that the nanoparticles are made of single crystal domain.

In general, we do observe that magnetite nanoparticle formation was detected above a threshold concentration of \( Fe^{3+}/Fe^{2+} \) ion pairs (50/25, note as Fe 50) as shown. Below the threshold concentration the formation of magnetite is hindered by the preferential binding of \( Fe^{3+} \) ions by the gelatin matrix. Moreover, above the threshold concentration, there are excess free \( Fe^{3+} \) ions for a higher amount of \( Fe^{3+}/Fe^{2+} \) ion pairs in the hydrogels. The free sufficient amount of \( Fe^{3+}/Fe^{2+} \) ions could co-precipitate for magnetite formation. It seems that \( Fe^{3+} \)-rich helices of the gelatin matrix serve as nuclear centers of the coprecipitation that leads to the alignment along the gelatin matrix. On the other hand, the size of the nanoparticles with a diameter of about 110 Å seems independent of the gelatin concentration and limited by the gel-cage size. Thus, both the gelatin matrix and the gelatin macromolecules influence the mineralization of the magnetite.
Figure 4.14. XRD patterns of magnetite @ gelatin hybrid dry-gels as a function of initial iron soaked concentration. The red dash line mark reveals the peaks from the gelatin structure while the green dash lines reveal the magnetite peaks.

4.4.4 Mechanism of Magnetite Mineralization in the Hydrogels

In Figure 4.15, we explored the kinetics of magnetite mineralization. Scattering curves of a 28 vol% D$_2$O of gelatin/magnetite composites, where magnetite is nearly the only visible part, were measured at different stages of mineralization from 0 s to 1200 s and show a strong increase in intensity (the reaction were stopped by quickly transfer the samples into pure water solution). During the initial period of reaction (< 200 s), with increasing the pH to 11 some iron(hydro)oxides precursor form and attach to the triple-helices of the gelatin matrix, and this makes the structure of the gelatin matrix visible by SANS (enhanced contrast). In between 120 s and 270 s the scattering curve changed significantly and immediately, indicating some structure formation in a fast way. There is no observation of step by step growth of the nanoparticle size indicating that the
mineralization may be directly from the surrounding iron oxide precursors. Formation of magnetite nanoparticles of ~133 Å in diameter ($D = 2\sqrt[3]{3}R_g$ for spherical samples) is detected after 270 s. After 1200 s, nanoparticles keep their fractal dimensionality of the dense phase of around 2.5.

Figure 4.15. In-situ SANS study of the magnetite mineralization kinetics in gelatin hydrogels in a mixture $D_2O/H_2O$ solvent of 28 vol.-% $D_2O$ and 72 vol.-% $H_2O$ (T = 20 ±2°C). The mineralization was started in $Fe^{2+}/Fe^{3+}$ loading hydrogels (18 wt% gelatin). The solid line represents a fitting result by eq. 4.2 (30 s, 120 s, 270 s) and Beaucage equation (High Q feature of 270 s, 420 s).
Figure 4.16. (A) Schematic representation of the magnetite mineralization kinetics in three steps. I) iron ion diffusion into the gel network, II) intermediate product formation and attachment on the gelatin molecules, III) magnetite formation from the intermediate products. (B) A representative structure for FeIII(OH)₃ coordination by collagen. Note that three carbonyl/hydroxyl groups are providing an O–Fe salt bridge via one short (0.23 nm) and two weaker (0.26 nm) contacts. (C) FeII(OH)₂ cluster coordination by collagen leading to distorted/incomplete octahedral coordination of FeII (the number of coordinating water molecules from the solvent varies from 0 to 2). Atom colors: Fe (yellow), O (red / green for solvent), H (white), N(blue) and C(grey). The pictures (B) and (C) are the simulation results from our collaboration partner in Erlangen (AG Prof D. Zahn) and adapted from our publication¹⁷⁰.

A possible mechanism of the coprecipitation process of the magnetite mineralization summarized in Figure 4.16 (A):

1) iron(hydro)oxides, Fe(OH)ₓ, form from the iron ions in the hydrogels and attached around the gelatin triple-helices; 2) nanoparticles are nucleated from those ion clusters. Finally, the magnetite nanoparticles of 138 (±40) Å in diameter are homogenously distributed along the gelatin chains (particles in a polymer matrix).

To characterize the interplay of gelatin and magnetite mineralization on the molecular scale, our collaboration group from Erlangen performed molecular simulation studies of Fe²⁺/Fe³⁺ and hydroxide ion association to a triple-helical (Gly-Pro-Hyp)ₙ peptide.¹⁷⁰ To allow direct comparison, the gelatin triple-helix as the collagen fragment and the simulation method is chosen in full analogy to earlier studies on calcium and phosphate ion association to collagen¹⁷⁷. From this, favorable association sites for both FeII(OH)₂ and FeIII(OH)₃ ion clusters were identified. Figure 4.6 (B) and (C) illustrate representative constellations as observed for each species. It is noteworthy, that both precursors to magnetite bind to gelatin triple-helix via hydrogen bonds and salt bridges without distorting the triple-helix. Instead, Fe(OH)ₓ binds to carbonyl and hydroxyl groups which
oxygen atoms tend to complete an octahedral coordination polyhedral for either Fe\(^{3+}\) and Fe\(^{2+}\) association.

The close interplay of Fe(OH)\(_x\) motifs and gelatin triple-helices as observed from molecular simulation hints at the suitability of collagen to bind iron and hydroxide ions (with the later only forming stable bonds in combination with iron ions). From this, we conclude triple-helices to act as nucleation sites to iron hydroxide aggregation, and thus intergrowth of gelatin and magnetite nanoparticles already at the precursor stage. Moreover, the TEM micrographs and the AFM of the final magnetite-gelatin composites indicate a structural alignment of the nanoparticles, which we attribute to magnetite nucleation along the gelatin matrix. This interplay of organic and inorganic components could give rise to hierarchical composites as observed for calcium phosphate – collagen-based biominerals\(^{178}\).

**Table 4.5.** Parameters of the gel-composites in D\(_2\)O/H\(_2\)O (D\(_2\)O Vol 28%) obtained from the kinetic measurement. The R\(_g\) and correlation length obtained from fitting results of equation 4.2 and 4.3.

<table>
<thead>
<tr>
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<th>30 s</th>
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<th>270 s</th>
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<tr>
<td>(\alpha)</td>
<td>2.5</td>
<td>2.4</td>
<td>1.9</td>
<td>2.4</td>
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<tr>
<td>(R_g/\text{Å})</td>
<td>-</td>
<td>-</td>
<td>50.8 ±0.5</td>
<td>45.1 ±0.5</td>
<td>58.3 ±0.5</td>
</tr>
<tr>
<td>(\xi/\text{Å})</td>
<td>7.9 ±3</td>
<td>31.1 ±1.1</td>
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### 4.4.5 Drying induced Reorganization in the Hybrid Materials

As a potential superhard material bio-inspired by teeth of Chitons, the gelatin-magnetite hydrogels need a further dehydration. The drying step may cause gelatin and magnetite reorganization, which are essential for the mechanical properties.

**Drying induced gelatin crystallization**

Figure 4.17 displays the time-resolved SAXS experiment on a drying induced gelatin crystallization. The measurements were performed in vacuum with the sample gelatin 18 wt% in D\(_2\)O and with recording the first data at 1min. The 3D plots of Figure 4.17 (A) show that in the first 40 min there are fast and significant changes in scattering intensity in the whole Q-range and later there are minor changes in the very high-Q range. The scattering curves are contributed by two structures of gelatin, the low-Q (Q < 0.3 Å\(^{-1}\)) structure of the gelatin network and the high-Q (Q > 0.3 Å\(^{-1}\)) of the molecular
scattering. In the high-Q there is a maximum appear at $Q_h$ where $d=2\pi/Q_h$ is the d-space of the gelatin crystals.

![Figure 4.17](image1.png)

**Figure 4.17.** Time-resolved SAXS study of the drying induced gelatin crystallization. The measurements were performed in vacuum with the sample gelatin 18 wt% in D$_2$O. (A) 3D plots of the TR-SAXS measurements. (B) Time-resolved SAXS scattering cross section against Q in a 2D plots.

![Figure 4.18](image2.png)

**Figure 4.18.** Time-resolved SAXS parameters of the dring induced gelatin crystallization.
Figure 4.18 are the plots of $R_g$ by fitting the low-Q data with the Beaucage equation (equation 4.2) and the d-space as a function of time. In the first 50 min, the value of $R_g$ rapidly increased which is correlated with a fast decrease of the d-space. These results demonstrate a growth and aggregation of gelatin triple-helices (increase the length and rod diameter) in conjugation with a crystallization of the triple-helices (decrease of d value). Finally, the drying induced reorganization of gelatin films results in a much thicker “bone” of network matrix within partly crystallized gelatin triple-helix dense phase.

**Drying effect on the Hybrid materials**

In X-ray scattering, the contribution of the gel matrix is less than 5%, which means that the scattering is dominated by magnetite. In Figure 4.19, the SAXS scattering of magnetite/gelatin thin films shows slightly correlation between the particles which means the observation of a structure factor. The effect of spatial correlation of the magnetite particles becomes more transparent from the X-ray scattering experiments discussed in Figure 4.19 showing scattering patterns of 12 and 18 w% magetitle-gel composites in wet and dry conditions. The solid lines represent the Beaucage fitting of the sample.

![Figure 4.19. SAXS intensity $d\Sigma/d\Omega(Q)$ versus scattering vector $Q$ for (made from gelatin 18 w%) wet and dry gel composites. The solid lines represent the fitting by equation 4.2.](image)

**Figure 4.19.** SAXS intensity $d\Sigma/d\Omega(Q)$ versus scattering vector $Q$ for (made from gelatin 18 w%) wet and dry gel composites. The solid lines represent the fitting by equation 4.2.
Superparamagnetic Magnetite Mineralization in Gelatin Hydrogels

Figure 4.20. SAXS intensity $d\Sigma/d\Omega(Q)$ versus scattering vector $Q$ for (made from gelatin 12 w%) wet and dry gel composites. The solid lines represent the fitting by equation 4.2.

For the 18 wt% wet gel sample we find particles of $R_g \approx 60 \text{ Å}$ from SAXS with a power law exponent of $\sim 2.75$ in the intermediate $Q$ regime. A Kratky plot in the inset of the Figure 4.19 exhibits a single distinct peak, which is typically indicative of globular structures. The peak at low $Q$ followed by an elevated baseline at high $Q$ indicates the particles with diameter $\sim 142 \text{ Å}$ attached to gelatin triple-helices. The diameter of the size larger than the SANS result of $\sim 108 \text{ Å}$ may be due to the small contribution from the gelatin network.

In contrast, the 12 wt% wet gels (Figure 4.20) show a smaller $R_g$ of $46.8 \text{ Å}$ and $Q^{-2.5}$ power law in the intermediate $Q$ regime. The diameter of the gel is $\sim 111 \text{ Å}$ indicating a smaller size which is close to the SANS results (Figure 4.8). This result is due to less gel matrix scattering contribution on the $R_g$, while SANS is nearly matching the organic contribution (in 28vol% of $D_2O$) and SAXS still have a small contribution on the gelatin. Kratky plots indicate that the two samples in wet status have a similar structure, i.e. particles attached to a chain.

The dry samples (12 wt%) show a much stronger scattering and correlation between magnetite because of their enhanced dense packing. At intermediate $Q$ one has $Q^3$ which at $Q = 0.23 \text{ Å}^{-1}$ transforms to $Q^4$ power law. The $Q^3$ behavior suggests a compo-
site mass fractal structure of magnetite or still some contribution from the gelatin. Kratky plots indicate that the particles in the dry sample show a more or less isolate-particle distribution within the dense matrix.

The correlation peak of the dry samples at $Q_m \sim 0.032 \text{ Å}^{-1}$ provides an average distance of the scattering particles of $\langle a \rangle \sim 243 \text{ Å}$ ($\langle a \rangle = 1.23 \times 2\pi/Q_m$)$^{179}$, which is larger than the $D = 2.38 \times R_g = 187 \text{ Å}$ of the particles. This means that the nanoparticles in the dry gels are densely packed. In the dry gels of 18 wt% gelatin shows average distance of $277 \text{ Å}$ with nearly the same $R_g$. In Figure 4.21 there are magnetite particles with the size $D \sim 82 \text{ Å}$ in the absence of gelatin which are densely packed with a $D$ to $\langle a \rangle$ ratio of $\sim 0.85$. This ratio for 12 wt% gelatin sample is 0.77 and 18 wt% gelatin samples of 0.68. Thus, the results indicate that the higher the concentration in gelatin hydrogels as the mineralization medium, the smaller the nanoparticles pack density. This strongly affects the materials mechanical properties.

Moreover, in all wet gels the average particle distances are larger than in the dry gel samples. In the case of wet 18 wt% gel sample, the average particle distance is $\sim 728 \text{ Å}$ while the cage of the hydrogels is only $\sim 260 \text{ Å}$. The result indicates that for one cycle of the mineralization, only a part of the gelatin cages were filled with magnetite nanoparticles. It is in agreement with AFM results (Figure 4.5) that only a part of the pores shows the magnetite nanoparticles. The SAXS results also suggest that the gelatin content has no significant influence on the size and distribution of the nanoparticles, i.e. the concentration of the gel matrix does not dominate the nanoparticles size since they have a similar average mesh size.
The SAXS scattering of a chiton tooth at different positions were shown in Figure 4.22 (A). It is clear that position P 8.5 (magnetite-chitin layer) shows the scattering mainly from the magnetite-chitin composites. While the other position shows the SAXS scattering including high amount of apatite (the core of the tooth). The SAXS data at P 8.5 shows two crossover Q points indicating more than three level structures while the scattering vector $Q \sim 0.25 \text{ nm}^{-1}$ reveals a structure with a typical size of $\sim 25 \text{ nm}$ corresponding to the magnetite nanoparticle size. Figure 4.22 (B) is a AFM image on the cross section of a chiton tooth. It is clear that the magnetite with the size of ten’s nanometer aggregate into large clusters. The results are in agreement with the SAXS data. WAXS result in Figure 4.22 (C) shows that the chitin matrix has a sharp peak. That is a conformation the chitin matrix is in crystalline phase.

Comparing the chiton tooth and gelatin-magnetite composites, they are both magnetite – organic matrix composites. However, in the chiton tooth, the magnetite nanoparticles are larger then that in gelatin-magnetite composites. Second, there are much higher magnetite loading ratios in the chiton tooth. Third, the organic matrix of the chiton tooth is mainly in highly crystalline phase. Those features make the chiton tooth showing
better mechanical properties than the gelatin-magnetite composites. However, we found the gelatin-magnetite composites are hard as hard as human bone.

Figure 4.22. (A) SAXS intensity $d\Sigma/d\Omega(Q)$ versus scattering vector $Q$ for chiton tooth at different positions. (B) AFM result on the cross section of a chiton tooth. (C) WAXS intensity $d\Sigma/d\Omega(Q)$ versus scattering vector $Q$ for chiton tooth.

4.5 Conclusions

In this chapter, the design of a bio-inspired method for room temperature precipitation of superparamagnetic magnetite is presented using the gelatin hydrogels as the preformed network, which closely mimics the mineralization from Chiton teeth. We have explored the roles of gelatin hydrogels on concentration as well as the bio-inspired magnetite mineralization mechanism by using small (SANS) and very small (VSANS) angle neutron scattering and SAXS in conjunction with TEM/AFM/XRD measurements.

In detail, we investigated the composites in $D_2O$ and 28 vol% of $D_2O$ in order to determine separately the roles of the organic and inorganic components (Figure 4.23). We investigated mineralization of a series of iron loaded gelatin precursors systematically varying the iron content ($c \ [Fe^{3+} / Fe^{2+}]$) from the ratios of 5/2.5 to 200/100 in units of
mM as well as the magnetite mineralization mechanism. The drying induced reorganization of the gels is also involved.

We aimed to provide novel insights establishing a direct relation between the spacing confinement of the gelatin matrix and the mineralized particles producing such highly and optimized sophisticated materials properties.

From the above investigation, general conclusions are given as follows:

1) The structure of the magnetite-gel composites were characterized with respect to gelatin as well as magnetite nanoparticles using SANS contrast matching, which is able to individually access the structure of each individual compound over the entire colloidal range as well as SAXS mainly visualizing magnetite.

2) SANS shows with respect to gel concentration an unchanged gelatin structure of average cage size larger than the magnetite nanoparticles. The size of the nanoparticles with a diameter of about 110 Å seems independent of the gelatin concentration and limited by the gel cage size. Both the gelatin matrix and the gelatin macromolecules influence the mineralization of the magnetite.

3) SANS shows no aggregation of magnetite in agreement with TEM and AFM. Magnetite particles itself show spatial correlations in SANS and SAXS due to excluded volume interaction, which particularly become strong for the dry samples.

4) Magnetite nanoparticle formation was detected above a threshold concentration of Fe$^{3+}$/Fe$^{2+}$ iron pairs. Below the threshold concentration the formation of magnetite is hindered by the preferential binding of Fe$^{3+}$ ions. It seems that Fe$^{3+}$-rich helices of the gelatin matrix serve as nuclear centers of the coprecipitation that leads to the alignment along the biopolymer fibrous structure.

5) A possible mechanism of the coprecipitation process of the magnetite mineralization is that the magnetite nanoparticles are nucleated from the preformed Fe(OH)$_x$ clusters. Such magnetite nanoparticles of ~110 Å in diameter are homogenously distributed and attached to the gelatin matrix (particles in a polymer matrix).

6) There is a reorganization process when drying the samples. The organic matrix crystallizes and the inorganic nanoparticles aggregate interact with each other.
Figure 4.23. Summary and schematic representation of the results of superparamagnetite mineralization in gelatin hydrogels in D₂O and D₂O/H₂O 28 V/V% obtained by SANS/VSANS.
5 Formation of Large Magnetite Nanoparticles in Gelatin Hydrogels

5.1 Introduction

5.1.1 Stable Single Domain Magnetite Particles

Magnetite ($\text{Fe}_3\text{O}_4$) is the most fundamental and important magnetic mineral on Earth. It is an important iron oxide polymorph having wide applications due to its high Curie temperature and the highest saturation magnetization among the natural iron oxides minerals.\textsuperscript{180}

![Diagram of magnetic regimes of magnetite as a function of their size (superparamagnetic, stable single domain, multi-domain).]

Figure 5.1. Magnetic regimes of magnetite as a function of their size (superparamagnetic, stable single domain, multi-domain).

In nanoparticle form, the magnetic behavior of a magnetite crystal depends upon the particle size and shape (Figure 5.1).\textsuperscript{181} The magnetite particles below a size threshold ($D_{\text{SPM}}$) of 30 nm exhibit superparamagnetic behavior at room temperature. Such particles do not possess a stable magnetic order due to thermal fluctuations induced random flipping of the magnetic moment.\textsuperscript{151, 153} Particle size above the threshold but smaller than 100 nm (DCR) are stable single domain (SSD) particles. The single domain nano-
particle is uniformly magnetized with all of the spins in alignment. Further size increase above 100 nm, the particles magnetically divides into multiple domains (MD).

As displayed in Figure 5.1, the coercivity is zero for superparamagnetic particles, but it increases in the stable single domain regime and shows a maximum with the development of multiple magnetic domains around $D_{\text{CR}}$. Thus, magnetite particles show the highest coercivity within the intermediate size of SSD typically ranging from 20 to 100 nm.

In nature, most of the biogenic magnetite particles have dimensions in a relatively narrow single domain regime. An amazing example is that single domain magnetite with the size in between 30 to 100 nm is produced intracellularly by a variety of magnetic bacteria in aquatic environments. The magnetite grain size has been interpreted as the result of natural selection on the magnetic properties of the crystals by organisms for their geomagnetic navigation.

### 5.1.2 Synthesis of Stable Magnetite Single Domain Nanoparticles

The formation of magnetite nanoparticles with controlled shapes and sizes can be achieved by numerous chemical methods, such as sol-gel syntheses, hydrothermal, synthesis microemulsions and coprecipitation methods. Among the various approaches to the synthesis of magnetite nanoparticles, the coprecipitation method is the most convenient and fast way to synthesize magnetite nanoparticles from an aqueous iron solution at room temperature. However, the method generally leads to small nanoparticles (< 20 nm) that are thus in the superparamagnetic regime with a broad size distribution. In contrast, natural magnetotactic bacteria synthesize 30-100 nm magnetite crystals aligned in chains, which are produced under aqueous, ambient conditions. Inspired from the magnetotactic bacteria, we want to synthesize the magnetite single domain nanoparticles in aqueous solutions and room temperature. Thus, the formation of large magnetic nanoparticles controlling the size and shape under mild conditions has become an attractive and necessary research goal.

Partial oxidation of Fe(II) ions at basic pH provides one way to produce single domain magnetite nanoparticles in aqueous solutions at room temperature. Such method involves the partial oxidation of Fe(II) ions at basic pH to ferric oxyhydroxide precursors and dehydration reaction of ferrous hydroxide and ferric precious to coprecipitate mag-
netite. The magnetite formation kinetics is controlled through the rate of oxidation, and more than an hour are needed to drive the reaction to completion. The partial oxidation method has led to the formation of different sizes (20 to 100 nm) and morphologies (hexagonal platelets, octahedral and isometric crystal) depending on the Fe$^{2+}$, OH$^{-}$ and oxidant ratio. The partial oxidation method has been successfully used to demonstrate the effect of biomolecular additives on the morphology and size of magnetite nanocrystals. Hence, it appears that the partial oxidation method becomes an attractive pathway to produce single domain magnetite nanoparticles in aqueous solution at room temperature.

### 5.1.3 The Aim of this Part

This part presents the adaptation of a bio-inspired method for the synthesis of large magnetite nanoparticles via a partial oxidation of ferrous ions in the aqueous, room-temperature conditions, which closely mimics the mineralization strategy taken by magnetotactic bacteria, using the gelatin hydrogel as the organic matrix/additive. We explore the role of gelatin hydrogels on bio-inspired magnetite mineralization by using small (SANS) and very-small (VSANS) angle neutron scattering in conjunction with TEM/XRD measurements. The scattering contrast of aqueous solution was adopted by the choice of D$_2$O content in order to determine the organic and inorganic components separately. We aimed to provide novel insights establishing a direct relation between the mineralization media/nucleation center of the gelatin matrix/additive and the mineralized particles producing highly and optimized sophisticated materials properties.

The following presented data was performed in collaboration with different groups in a Priority program project of the DFG (SPP 1569 Generation of multifunctional inorganic materials by molecular bionics). Samples synthesis and parts of non-scattering characterization are closely collaborated with Maria Siglreitmeier (a Ph.D. student in AG Cölfen, Uni Konstanz). Parts of the presented results in this chapter were adapted from the following publications:

Formation of Large Magnetite Nanoparticles in Gelatin Hydrogels


5.2 **Experimental Section**

5.2.1 **Materials**

Materials: The following commercially available chemicals were purchased and applied in the synthesis without further purification: FeSO$_4$·7H$_2$O (Sigma-Aldrich), H$_2$SO$_4$ (Sigma-Aldrich), KNO$_3$ (Sigma-Aldrich), KOH (Sigma-Aldrich), D$_2$O (99.8% D, ARMAR Chemicals), Gelatin Type B (~225 Bloom, Sigma-Aldrich), 4-chloro-m-cresol (Fluka), Methanol (VWR). For the preparation of the reactant solutions double-distilled and deionized (Milli-Q) water was used. All iron including solutions were degassed with argon before use.

5.2.2 **Synthesis of Gelatin Hydrogels**

Different amounts of gelatin were allowed to swell in water for 24 hours at 5 ±1°C. Homogeneous solutions were prepared by heating the gels for 2 hours at 45 °C. In each case, 10 mL of solution is filled into a glass dish (D =11 mm) and allowed to form a hydrogel film with a thickness ~1 mm. To avoid decomposition by bacteria, a 5 wt% solution of 4-chloro-m-cresol in methanol was added (0.15 mL per 1 g of dry gelatin). All the samples for SANS-VSANS measurement were prepared in D$_2$O or a mixture of D$_2$O/H$_2$O.
5.2.3 Magnetite Mineralization in Gel Matrices

In-situ mineralization of magnetite nanoparticles in gelatin hydrogel was carried out via partial oxidation of FeSO₄ in gelatin hydrogels. Each gelatin hydrogel film sample was introduced into 50 ml of aqueous FeSO₄ (by adjusting pH = 1.5 by H₂SO₄) and soaked for 96 hours at 5 (±1)°C. The iron (II)-loaded gel film precursors were washed with water and placed in 0.1 M KOH and 0.5 M KNO₃ solution from 1 min to 24 h. The resulting magnetite/gelatin composite films were washed 3 times with MQ water respectively. The solvents of H₂O were changed to D₂O or a mixture of D₂O and H₂O for all the SANS characterization, respectively. The detailed experimental steps are schematically presented in Figure 5.2.

Figure 5.2. Schematic representation of experimental steps. (1) Synthesis of Gelatin Hydrogels. (2) The gelatin hydrogel films. (3) Magnetite mineralization in the hydrogel matrices.

5.2.4 SANS and VSANS Experiments

Small-Angle Neutron Scattering (SANS) and Very-Small Angle Neutron Scattering (VSANS) experiments were carried out at, respectively, the KWS1 and KWS3 diffractometers of the JCNS outstation at Heinz Maier-Leibnitz Zentrum (MLZ) in Garching, Germany. Four configurations were used at KWS1, namely the sample-to-detector (SD) distances of 1.3, 2, 8 and 20 m, the corresponding collimation length of 8 and 20 m, and a wavelength of 7 Å (Δλ/λ = 10%). These settings allowed covering a Q-range from 0.002 to 0.35 Å⁻¹. The scattering vector Q is defined as $Q = 4\pi\lambda\sin\theta$ with the scattering angle 2θ and the wavelength λ. A two-dimensional position sensitive detector was
used to detect neutrons scattered from the sample solutions. Films characterized by SANS and VSANS were hydrated in D$_2$O or mixture of D$_2$O/H$_2$O and placed in a sample cell between two quartz windows with a path length of 1 mm. Plexiglas was used as a secondary standard to calibrate the scattering intensity in absolute units at KWS1 and direct beam method at KWS3. The data correction and calibration were performed using the software QtiKWS$^{96}$. Fitting the SANS data was done using software modules provided by JCNS QtiKWS and NIST Igor$^{127}$ analysis packages. In order to cover the broader length scale of the network structure, very-small angle Neutron scattering (VSANS) experiments were carried out at the KWS3 diffractometer using the parabolic mirror as an optical element, and covering the smaller Q range from 0.0001 to 0.002 Å$^{-1}$.

Some of the SANS data were measured at SANS II at Paul Scherrer Institute (PSI) in Villigen, Switzerland. The sample-to-detector distances were 1 and 5 m, the corresponding collimation lengths 4 and 5 m, and the wavelength 5.2 Å. Water was used as a secondary standard to calibrate the scattering intensity in absolute units. These settings allowed covering a Q-range from 0.01 to 0.35 Å$^{-1}$.

Fitting the SANS data was done using software modules provided by JCNS QtiKWS$^{96}$ and NIST Igor$^{127}$ analysis packages. Some expression and models for the hybrid materials as following:

**The Beaucage Expression**

The Beaucage empirical expressions are able to reasonably approximate the scattering from many different types of particles, including fractal clusters, random coils (Debye equation), ellipsoidal particles, etc.$^{103}$ Beaucage expression is given according to:

$$
\frac{d\Sigma}{d\Omega}(Q) = \frac{d\Sigma}{d\Omega}(0) \exp(-u^2/3) + P_a \left[ \text{erf} \left( u / \sqrt{6} \right) \right]^3 / Q^\alpha
$$

(5.1)

representing a combination of Guinier’s and Porod’s laws describing the scattering at low and large Q, respectively. More quantitatively both approximations are valid for the parameter $u = R_gQ$ smaller or larger than 1, $u$ representing the product of radius of gyration $R_g$ and scattering vector $Q$. Guinier’s law has the shape of a Gaussian function whereas for $Q$ larger than 1/$R_g$ ($u>1$) a power law according to $d\Sigma/d\Omega(Q) = P_a Q^{-\alpha}$ is often observed.
In this chapter, the scattering curves from the inorganic minerals were fitted with Beaucage expression to generate the size and dimensions of a sample.

**The Correlation Length Model**

The correlation length model developed by B. Hammouda\(^\text{104}\) is made of two terms. The first term describes Porod scattering from clusters (exponent = \(n\)) at small \(Q\) and the second term is a Lorentzian function describing scattering from polymer chains (exponent = \(m\)) at larger \(Q\). The second term \(C/[1+(Q\xi)^m]\) characterizes the polymer/solvent interactions and therefore the thermodynamics. The two multiplicative factors \(A\) and \(C\), the incoherent background BKG and the two exponents \(n\) and \(m\) are used as fitting parameters.\(^\text{79, 104}\)

\[
\frac{d\Sigma}{d\Omega} (Q) = \frac{A}{Q^n} + \frac{C}{1+(Q\xi)^m} + BKG
\]  
(5.2)

In the case of the polymer clusters, the first term \(A/Q^n\) becomes a key parameter to evaluate the clustering strength. In this chapter, the composites in D\(_2\)O corresponding to the organic matrix structures are fitted with correlation length model to generate the correlation length and to evaluate the clustering strength.

**5.2.5 Transmission Electron Microscopy (TEM)**

For TEM analysis, a Zeiss Libra 120 operating at 120 keV and a JEOL JEM-2200FS operating at 100 keV were used, respectively. For material characterization, a drop of a diluted dispersion of nanoparticles extracted from the hydrogels was placed on formvar carbon coated copper grids and left to dry on a filter paper for measurements.

**5.2.6 Powder X-ray diffraction (XRD)**

X-ray diffraction (XRD) patterns on dried composite films were collected using a PANalytical X’Pert X-ray powder diffractometer equipped with a Cu K\(\alpha_1\) radiation source (\(\lambda = 1.5406\) Å).

**5.2.7 Superconducting Quantum Interference Device (SQUID)**

Magnetization measurements were carried out by using a quantum design SQUID 5 T magnetic properties measurement system (MPMS). For measurements, dried samples were introduced into gelatin capsules and magnetization loop measurements at 2 K and
Formation of Large Magnetite Nanoparticles in Gelatin Hydrogels

293 K were performed. In addition zero-field-cooled and field-cooled curves were obtained by applying 0.01 T and heating or cooling the sample. The measurements were done by Maria Sigleitmeier at the University of Konstanz.

5.3 Results and Discussion

The synthesis process of large magnetite-gelatin composites followed the established in-situ mineralization method in chapter 4 and was modified as shown in Figure 5.2. The Fe\(^{2+}\) ions were used as the only iron source and the mineralization under base conditions (KOH) was performed in presence of the oxidising additive, KNO\(_3\). The experiments included two key steps: 1) Ferrous salts loading in the gelatin hydrogels. 2) large magnetite mineralization by the partial oxidation of ferrous ions at pH \(\approx 11\).

5.3.1 The Iron(II)-loaded Gelatin Precursors

![Figure 5.3](image)

**Figure 5.3.** (a) SANS-VSANS scattered intensity versus scattering vector Q for ironII-loaded gelatin in D\(_2\)O (T = 20 ±2°C). The solid line represents the best fit by equations 5.1 (Beaucage equation, for Q > 0.01 Å\(^{-1}\)) and 5.2 (correlation length model, for Q > 0.01 Å\(^{-1}\)). Parameters from SANS-VSANS pattern of iron(II)-loading and iron-free gelatin samples are listed in the inset table.

To form large magnetite-gelatin gel composites by partial oxidation of Fe\(^{2+}\) ions, gelatin hydrogel films were first soaked in an aqueous iron ion solution. Combined SANS and VSANS data shown in Figure 5.3 can be used to depict structure changes in the iron II
loaded hydrogel networks as the magnetite mineralization precursor. We observe in Figure 5.3 that the curves are slightly varying, but essentially constant for the iron loaded gel precursors with Fe\(^{2+}\) ions (Gel-Fe\(^{2+}\)) or without Fe\(^{2+}\) ions (Gelatin-Gel 12 wt\% in D\(_2\)O). SANS/VSANS results show that Fe\(^{2+}\) ions have no influence to gelatin structure. The iron loading gel has nearly the same structure as the iron-free gelatin hydrogels. Also, absence of gelatin degradation by highly acid loading concentration is proven.

### 5.3.2 Large Magnetite Formation in the Hydrogels

Large magnetite nanoparticles were formed in the presence of a gelatin network after immersing the iron II loading gel precursor (gelatin soaked in a 200 mM Fe\(^{2+}\) or 300 mM Fe\(^{2+}\) contained solutions) into a KOH (0.1 mol L\(^{-1}\), pH \(\approx 11\)) and KNO\(_3\) (0.1 to 0.5 M) solution. The partial oxidation method leads to the formation of large sizes (20 to 150 nm) and morphologies (hexagonal platelets, octahedral and isometric crystal) depending on the Fe\(^{2+}\), OH\(^-\), oxidant ratios, temperature and cations.\(^{185,186}\) In the thesis, we are focus on several parameters which influence on the magnetite particle size, shape and formation kinetics. For another, the roles of the organic matrix is essential to discuss.

**General properties**

The resulting magnetite-gelatin hybrid gels with a deep dark color, strong magnetic response and soft texture formed after few hours. The magnetic properties of the synthesized large magnetite-gelatin composites are investigated by magnetization hysteresis curves as well as field cooled and zero field cooled experiments which were conducted using a super quantum interference device (SQUID).\(^{187}\) Figure 5.4 (A) presents the temperature dependence of magnetization (M-H) measured at 2 K and 293 K. As one can see clearly from the figure and its inset, at 2 K the gelatin-Fe\(_3\)O\(_4\) composites show typical ferrimagnetic behavior with a distinct coercivity (\(H_c \sim 250\) Oe), while the coercivity is around 36 Oe at 293 K.

Figure 5.4 (B) shows the temperature dependence of zero-field-cooled (ZFC) and field-cooled (FC) magnetization (M-T) of the magnetite-gelatin composites. The FC magnetization slightly increases with increasing the temperature indicating strong interparticle interactions where the interactions frustrate the magnetic order.\(^{188,189}\) The ZFC curve of the dry composites sample which does not show TB under room temperature identifies
the ferrimagnetic properties of the material. Furthermore, the bending in the ZFC curve around 25 K indicates the Verwey transition temperature.

**Figure 5.4.** A) Temperature dependence of magnetization (M-H) measured at 2 K and 293 K. B) FC and ZFC curves plotted as a function of temperature. The magnetic result data is a representation from Maria S.\textsuperscript{187}.
Figure 5.5. TEM results of the formed large magnetite nanoparticles. (A) Composites prepared from an iron source of 0.2 M Fe$^{2+}$ and (B) repeated mineralization process for 4 reaction cycles. (C) HR-TEM image of a composite sample after one reaction cycle. (iron II source of 0.2 M Fe$^{2+}$) and the FFT image (D). The data is a representation and adapted from Maria S.187.

Typical TEM results (adapted from Maria S.187) obtained from a composite sample loaded with 0.2 M FeSO$_4$ solution in Figure 5.5 (A) show spherical particles with a diameter 500 (±150) Å. The size is fixed in a stable single domain regime showing ferrimagnetic properties which is in agreement with the magnetic measurements. When repeating the mineralization in 4 reaction cycles (means gelatin long time swollen in water), result reveals chain-like arrangement of the nanoparticles due to the inter-particle dipolar interaction. HRTEM image in Figure 5.5 (C) shows a much clearer morphology of the spherical particle with a size of ~450 Å and a rough surface. In
Figure 5.5 (D). The Fourier analysis (FFT) indicates that the nanoparticle possesses an [112] orientation, with lattice fringes covering the whole particle, which can be indexed based on the crystal structure of magnetite (Fd3m, a = 8.3969 Å). Moreover, in Figure 5.5 (C) the nanoparticle seems made of smaller subunits with orientation due to their single crystal like scattering in Figure 5.5 (D). However, from the TEM results one thing is confusing that near all samples obtained from this method has a size larger than 300 Å. Thus the particle size is larger than the gelatin hydrogel cage size we mentioned before from the SANS data that can explain the observed pores and the presence of smaller subunits.

5.3.3 SANS Contrast Variation Studies of the Composites

In order to further clarify the structure of the gelatin-nanoparticle hybrid material, we performed SANS contrast variation experiments, which allowed to independently explore the inorganic nanoparticle structure as well as the gelatin gel network. Contrast variation SANS experiments became a standard method as pointed out and applied also in cognate disciplines such as biomineralization. Especially the question from TEM results why magnetite has a size larger than the cage size of the gelatin hydrogels was to special interest here. Figure 5.6 (A) and Figure 5.7 display SANS scattering patterns of composites dissolved in an aqueous mixture of 28 vol% D₂O and in pure D₂O and matching the scattering of gelatin and magnetite, respectively. The yellow open circles in Figure 5.6 (A) represent the scattering of the composites in a 28 vol% D₂O aqueous solution, which matches the gelatin scattering and visualizes the magnetite nanoparticles. There is weakly enhanced scattering as high as the scattering of composites in D₂O in the small Q-range, which might caused by a scattering of the magnetic nanoparticle ‘chains’ (Figure 5.5 B). The scattering in the intermediate Q-range caused from individual nanoparticles of \( R_g = 460 (±1) \) Å shows a \( Q^{-3} \) power law at intermediate Q which might indicate a mixture of volume fractal and surface fractal. The spherical diameter \( D \) of the magnetite particles can be estimated as \( D = 1190 (±3) \) Å (\( D = 2\sqrt{3}R_g \)). This value is in agreement with the result obtained from TEM (diameter range from 300 to 1500 Å). The SANS result is slightly larger than the TEM may be caused by the fractal structure of the particles and the large detector area statistical results. That is also a reason for the size of the particle being much large than the
cage of the hydrogels (~260 Å) indicating that the mineralization process incorporates
the gelatin network inside the large magnetic particles. Thus, all the large particles have
a “polymer in particle” structure, as it shown in the inset of Figure 5.6 (a). The roles of
gelatin in the particles is similar to some organic matrix in biominerals and bio inspired
minerals\textsuperscript{67}, where the organic matrix hybrid with the inorganic minerals shows im-
proved mechanical properties. Further, the scattering curve in the high Q has a cross
over Q\textsubscript{c} (~0.12 Å\textsuperscript{-1}) reflects a small structure with a size less than 50 Å. This small
structure may come from the small building unit or the repeat of the reaction regrowth
of new particles.

In D\textsubscript{2}O, when gelatin is the only visible part, the scattering at high Q (Q > 0.02 Å\textsuperscript{-1})
delivers a Q\textsuperscript{-1} rod-like power law scattering which is similar to the pure gelatin of 12
wt\% in D\textsubscript{2}O. For the gelatin hydrogels in the very low-Q regime, the scattering curve
has a trend to flat Guinier regime which yields a very large radius of gyration of R\textsubscript{g} =
1580 (±40) Å of the large clusters. In contrast, in the low Q regime magnetite/gelatin
hydrogels in D\textsubscript{2}O show a Q\textsuperscript{-2.8} power law scattering corresponding to a mass fractal
structure (branched structure). The scattering is very similar to the Fe\textsuperscript{3+} loaded hydrogel
precursors (shown in the inset). Thus, the gelatin structure is an irreversible return to the
original structure during the magnetite mineralization due to the irreversible aggregation
of large clusters. However, after the mineralization, the gel structure still keeps the cage
like networks without any collapse.
Formation of Large Magnetite Nanoparticles in Gelatin Hydrogels

Figure 5.6. A typical sample synthesized by the partial oxidation method, by which 10 wt% gelatin hydrogels with FeSO₄ (0.3 M) are treated in a KOH (0.1 M), KNO₃ (0.25 M) solutions in 4 reaction cycles. (a) SANS-VSANS scattering intensity against Q measured in 28 vol% D₂O, the solid line is the best fitting by equation 5.1. (b) TEM image of the large magnetite nanoparticles-gelatin composites.
Formation of Large Magnetite Nanoparticles in Gelatin Hydrogels

**Figure 5.7.** SANS-VSANS scattering pattern of the gelatin-magnetite composites in pure D$_2$O. The sample was prepared from 10 wt% gelatin hydrogels with FeSO$_4$ (0.3 M) and treated with a KOH (0.1 M), KNO$_3$ (0.25 M) solutions in 4 reaction cycles. The gelatin hydrogels 12 wt% in D$_2$O was plotted for comparison. The solid lines are the best fitting by equation 5.1 and 5.2 as well as the power law for the composites at very low $Q$.

5.3.4 The Influence of Oxidant and Iron Source Concentration

The partial oxidation method has led to the formation of large sizes over 20 nm and various morphologies depending on the $\text{Fe}^{2+}$, $\text{OH}^-$, oxidant ratios and the temperature.$^{185, 186}$ Here we discuss the oxidant and iron source concentration influence on the magnetite size, and shape.
Formation of Large Magnetite Nanoparticles in Gelatin Hydrogels

Figure 5.8. Sample synthesized by the partial oxidation method, by which 10 wt% gelatin hydrogel with FeSO₄ (0.2 M) are treated in KOH (0.1 M), KNO₃ (0.25 M and 0.5 M) solutions in 4 reaction cycles.

Figure 5.8 represents the scattering of the composites in a 28 vol% D₂O aqueous solution, which matches the gelatin scattering and visualizes the magnetite nanoparticles. Two samples mineralized from different KNO₃ ratios were compared in Figure 5.8. For the sample synthesized by using 0.5 M KNO₃, noted as 0.5 M, the scattering in the intermediate Q-range is caused from individual nanoparticles of $R_g \approx 310 \text{ Å}$ showing a $Q^{-3}$ power law at intermediate Q which might indicate a mixture of volume fractal and surface fractal. The spherical diameter $D$ of the magnetite particles can be estimated as $D \approx 800 \text{ Å}$ ($D = 2\sqrt{5/3}R_g$). Higher Q shows a $Q^{-3.8}$ power law indicating a dense structure in the particles. There is a crossover for the power law transit to $Q^{-1.5}$ corresponding to a structure on the length scale of 60 Å possibly from the small building units of the large structure. Thus, the sample 0.5 M may be composed of densely packed small building particles connecting the gelatin network which causes the fractal structure of the composites. The structure is the so-called “matrix in particle” structure. The sample 0.25 M prepared from less oxidant indicates the smaller size of $R_g \approx 210 \text{ Å}$ and diameter of $\sim 540 \text{ Å}$. The similar high Q scattering means that the two samples have
similar small subunit structure. Thus with increasing the KNO₃ concentration, the size of the particles is significantly increased.

Figure 5.9. Sample synthesized by the partial oxidation method, by which 10 wt% gelatin hydrogel with FeSO₄ (0.2 M and 0.3 M) are treated in KOH (0.1 M), KNO₃ (0.5 M) solutions in 4 reaction cycles.

Figure 5.9 represents the scattering of the composites in a 28 vol% D₂O aqueous solution, which matches the gelatin scattering and visualizes the magnetite nanoparticles. Two samples mineralized from different iron II concentrations are compared in Figure 5.9. For the sample synthesized by using 0.3 M Fe³⁺, note as 0.3Fe, the scattering in the intermediate Q-range is caused by individual nanoparticles of \( R_g \approx 349 \, \text{Å} \) showing a \( Q^{-3.3} \) power law at intermediate Q which might indicate a mixture of volume fractal and surface fractal. The spherical diameter D of the magnetite particles can be estimated as

\[
D \approx 900 \, \text{Å} \quad (D = 2\sqrt[3]{5/3}\, R_g)^{70.}
\]

There is a crossover for the power law to \( Q^{-1.5} \) corresponding to a structure of the length scale of 60 Å possibly from small building units of the large structure. Thus, the sample structure is similar to the structure discussed in Figure 5.8 which shows the so-called “matrix in particle” structure. The sample 0.2M Fe prepared from less iron II concentration indicates the size of \( R_g = 310 \, \text{Å} \) and diameter of \( \approx 800 \, \text{Å} \). However, the size of the two samples is not very different. The high Q scattering shows a scale factor of 2 between two samples indicating that the higher iron con-
Formation of Large Magnetite Nanoparticles in Gelatin Hydrogels

centration results in a high number density of nanoparticles. The results are in constant to a literature report in a solution that with increasing iron II concentration a small amount of excess iron II results in relatively large (0.4-1.1 µm) spherical particles.\textsuperscript{190, 191} However, that may be caused by a different growth environment in between solution and hydrogels, where the hydrogel limits the diffusion and makes a big difference in the mineralization conditions.

5.3.5 Large Nanoparticle Mineralization Kinetics

In Figure 5.10, we explored the kinetics of magnetite mineralization. Scattering curves of a 28 vol% D\textsubscript{2}O solutions of gelatin/magnetite composites, where magnetite is nearly the only visible part, were measured at different stages of mineralization from 3 min to 7 h and show a strong increase in intensity (The coprecipitation process was stopped by quickly transfer the samples into pure water solution, the samples then will store more than 24 hours before the measurements). The scattering curves were fitted with the Beaucage equation (Eq. 5.1) and the resulting parameters were plotted in Figure 5.11.

![Figure 5.10](image)

\textbf{Figure 5.10.} In-situ SANS study of the magnetite mineralization kinetics in the presence of gelatin hydrogels (18 wt\%) in a mixture D\textsubscript{2}O/H\textsubscript{2}O solvent of 28 vol.-% D\textsubscript{2}O and 72 vol.-% H\textsubscript{2}O (T = 20 ±2°C). The inset is a gelatin large particle shape construction result from the scattering data by a rapid \textit{ab initio} shape
Formation of Large Magnetite Nanoparticles in Gelatin Hydrogels
determination program DAMMIF\textsuperscript{134}. The solid line represents the fitting results by the Beaucage expression (equation 5.1).

The scattering mainly shows two characteristic time steps. Within the initial 10 min, there is a continuous growth of the particles with shape changing from rod-like structure ($Q^1$ scattering) to spherical particles with diameter $\sim 1500$ Å ($R_g \approx 586$ Å) and mass fractal structure ($Q^3$ scattering). The fractal structure comes from the presence of the polymer matrix in the large particles (matrix in particle structure).

Above 30 minutes up to 7 hours there is no significant increased of the particle size but only a reorganization of the large magnetite particles via a magnetic dipole to an array of one-dimensional aligned particle chains ($Q^1$ scattering, Figure 5.11). Such particle alignments also found in magnetotactic bacteria in which the magnetic particles are along a protein chain\textsuperscript{192}.

![Figure 5.11](image.png)

**Figure 5.11.** Parameters obtained from the TR SANS-VSANS results. On the right is the possible magnetite formation mechanisms.

In order to probe the formed mineral phase, in Figure 5.12, XRD spectra of magnetite-gelatin composite films as a function of mineralization time were presented. A typical XRD spectrum of pure dry gelatin displays two wide peaks, at $2\theta = 7.7^\circ$, $\sim 19^\circ$. These peaks depict the diameter of the triple helix (repeat distance of 11.7 Å), the amorphous halo, respectively\textsuperscript{138}. In contrast, the intensity of gelatin peaks indicating triple-helical regions ($2\theta = 7.7^\circ$) were decreased with the mineralization time. However, the triple
helical peak does not appear in the hybrid gels. Therefore, it was proven that the mineralization in the gelatin matrix enabled the suppression of gelatin chain helicity, which is crucial for the mechanical properties of a gelatin network with defined structure-property relationships. Above the mineralization time of 30 min, XRD sharp peaks at 30.1°, 35.5°, 43°, 57°, and 62.5° match the typical diffraction patterns observed for magnetite (JCPDS Card No. 19-0629) as showing at the bottom of Figure 5.12. At the initial time of 2 min, no magnetite phase is found, but several sharp peaks may contribute from lepidocrocite (γ-FeOOH 2θ = 26.1°, 53.9° and 61° JCPDS Card No. 44-1415)\textsuperscript{193}, which is a rod-like mineral. The lepidocrocite phase formed from a transformation of green rust (unstable intermediate iron compound).\textsuperscript{193}

![XRD patterns of magnetite-gelatin dry gels as a function of mineralization time.](image)

**Figure 5.12.** XRD patterns of magnetite-gelatin dry gels as a function of mineralization time.

In addition, we have investigated the ferrimagnetic magnetite mineralization in a gelatin matrix via partial oxidation method in the presence magnetic field by VSANS (Figure 5.13). Samples in the middle stage of mineralization time of ~25 min were measured
with (0.4 T) and without magnetitic filed in D$_2$O 28 vol%, where gelatin is invisible. Figure 5.12 shows a dramatical decreased of the scattering intensity by a factor of ~5 in the magnetic field. The changing of the intensity is due to the magnetic field which separates the large magnetic particles thus only small amount of non-magnetic particles is detected. The results suggest that the ferrimagnetic magnetite mineralization is starting from the non-magnetic iron oxide particles with the slow transformation of the mineral phase into aligned magnetite larger nanoparticles.

![Figure 5.12](image)

**Figure 5.12.** VSANS study of the magnetite formation in the presence of gelatin hydrogels in 28 wt% D$_2$O (T = 20 ±2°C) with and without magnetic field.

When comparing the SANS-VSANS and XRD results, we can conclude a possible mechanism for the large particle mineralization via the partial oxidation method in the hydrogels. By placing the hydrogels into solutions of KOH and KNO$_3$, unstable amorphous ferrous hydroxides were immediately precipitated. After 2 min hydrogels appear dark green color, while SANS results show rod-like particle formation with a $R_g$ large than 320 Å. The rod-like particles can be identified as Goethite and lepidocrocite which are found in the XRD results. The products appear at the time stage due to the increasing pH still much lower than 11. After 10 min, larger magnetite particulates are present in the XRD measurements. The particle size has a significant growth up to around 1 h, where the particles have a $R_g$ of ~ 680 Å and diameter of ~ 1760 Å as well.
as a fractal-matrix in particle-like structure. X-ray results confirm its magnetite phase and a large crystal size. The further reaction has no significant increase of the size but leads to a linear alignment due to a magnetic moment. This particle alignment means the magnetic interaction force overcomes the gelatin molecule interaction while it is not found during the small particle formation cases. During the large magnetite particle formation, there are only Fe$^{2+}$ ions involved as the iron source in this large particles formation process. Ferrous ions have a weak interaction with gelatin matrix, which make it more-or-less like a homogenous mineralization in a gel network.

### 5.4 Conclusion

In this chapter, we presented the design of a bio-inspired method for room temperature formation of larger magnetite nanoparticles in the presence of a gelatin hydrogel, which closely mimics the mineralization strategy taken by magnetotactic bacteria. We have explored the role of gelatin hydrogels as well as the bio-inspired magnetite mineralization mechanism during the large magnetite particle formation by using small (SANS) and very-small (VSANS) angle neutron scattering in conjunction with TEM/XRD measurements.

In detail, we investigated the composites in D$_2$O and 28 vol% of D$_2$O (SANS contrast variation experiment) in order to determine separately the roles of the organic and inorganic components (Figure 5.14). Some parameters influence mineralization were also discussed. We measured the samples at different mineralization time stages to explore the mineralization mechanisms.

From the above investigation, general conclusions are given as follows:

1) The structure of the magnetite-gelatin composite gels was characterized with respect to gelatin as well as magnetite nanoparticles using SANS contrast matching, which is able to individually access the structure of each individual compound over the entire colloidal range.

2) SANS-VSANS shows an unchanged gelatin network structure of average 3D cage size smaller than the magnetite nanoparticles. The size of the large nanoparticles with diameters between 300 to 1500 Å seem independent of the gelatin concentration and include the gelatin matrix to form a “matrix in particle” structure. The Fe$^{2+}$ ions show a
weak interaction with the gelatin matrix resulting a homogenous-like mineralization which makes it possible to growth large particles in the gel matrix.

3) Although gelatin hydrogels have no direct effects on controlling the nanoparticles size, the gel networks have a contribution to the ion diffusion thus showing a buffer role on the instant pH and ion ratios, which will influence the mineralization kinetics.

4) A possible mechanism of the large magnetite nanoparticles formation is that the magnetite nanoparticles formed via a rod-like intermediate precipitate formation and transition to large magnetite particles over 30 minutes. Both magnetic (magnetite) and non-magnetic phase (goethite, lepidocrocite and amorphous ferrous hydroxides) were detected during the mineralization. Further reaction for several hours results in a highly swollen gelatin network which allows particle alignment by the magnetic dipoles.

Figure 5.14. Summary and schematic representation of the results of large magnetite nanoparticles formation in the presence of gelatin hydrogels in D₂O and D₂O/H₂O 28 V/V% obtained by SANS/VSANS.
6 Two Step Magnetite Mineralization in Gelatin Hydrogels

6.1 Introduction and Aim
The biominerall magnetite (Fe₃O₄) is found in a wide range of organisms, from bacteria to higher vertebrates such as birds and fish. Chiton teeth are one example which is extremely wear resistant through a hybrid design of magnetite nanoparticles embedded in a polysaccharide-protein gel matrix.⁴⁸,⁵² The fully mineralized chiton tooth consists of a magnetite/protein-polysaccharide hybrid shell displays remarkable functional properties including the highest reported hardness among known biominerals. The proteins and pre-formed polysaccharides fibrous hydrated networks buried within the teeth that provide a spatially confined template for the growing tooth's magnetite shell with defined geometries. During mineralization, the magnetite in chiton tooth is not formed in a one-step process but via a solid phase transition from ferrihydrite to magnetite.⁵² The two-step or multistep mineralization is normal in nature because the sophisticated process can avoid higher energy barriers providing in mild mineralization conditions.⁴⁴

In Chapter 4 we reported a co-precipitation method to produce small magnetite nanoparticles in a gelatin hydrogel matrix. In Chapter 5 we reported a partial oxidation pathway to form large magnetite nanoparticles within a gelatin matrix. The different methods have their particular advantages. In this chapter, we want to combine the two methods and to observe the presence of small nanoparticles in a gel matrix to investigate whether in the second step the particle will grow individually or grow by using the small particles as seeds.

6.2 Experimental Section

6.2.1 Materials
Materials: The following commercially available chemicals were purchased and applied in the synthesis without further purification: FeSO₄·7H₂O (Sigma-Aldrich), H₂SO₄ (Sigma-Aldrich), NaNO₃ (Sigma-Aldrich), NaOH (Sigma-Aldrich), KOH (Sigma-
Two step Magnetite Mineralization in Gelatin Hydrogels

Aldrich), D$_2$O (99.8% D) (ARMAR Chemicals), Gelatin Type B (∼225 Bloom, Sigma-Aldrich), 4-chloro-m-cresol (Fluka), Methanol (VWR). For the preparation of the reactant solutions double-distilled and deionized (Milli-Q) water was used. All iron including solutions were degassed with argon before use.

6.2.2 The two-step Mineralization of Magnetite Nanoparticles

In-situ mineralization of magnetite nanoparticles in gelatin hydrogels was carried out via two steps precipitate of iron ions in gelatin hydrogels. The method combines the coprecipitation (chapter 4) and the partial oxidation pathway (chapter 5) in a two-step process. Details of every step can be found in chapter 4 and 5.

6.2.3 SANS and VSANS Experiments

Small-Angle Neutron Scattering (SANS) and Very-Small Angle Neutron Scattering (VSANS) experiments were carried out at, respectively, the KWS1 and KWS3 diffractometers of JCNS outstation at Heinz Maier-Leibnitz Zentrum (MLZ) in Garching, Germany.$^{92}$ Four configurations were used at KWS1, namely the sample-to-detector (SD) distances of 1.3, 2, 8 and 20 m, the corresponding collimation length of 8 and 20 m, and a wavelength of 7 Å ($\Delta\lambda/\lambda = 10\%$). These settings allowed covering a Q-range from 0.002 to 0.35 Å$^{-1}$. The scattering vector Q is defined as $Q = 4\pi/\lambda \sin \theta$ with the scattering angle 2$\theta$ and the wavelength $\lambda$. A two-dimensional position sensitive detector was used to detect neutrons scattered from sample solutions. Films characterized by SANS and VSANS were hydrated in D$_2$O or a mixture of D$_2$O/H$_2$O and placed in a sample cell between two quartz windows with a path length of 1 mm. Plexiglas was used as a secondary standard to calibrate the scattering intensity in absolute units at KWS1 and direct beam method at KWS3. The data correction and calibration were performed using the software QtiKWS.$^{96}$ Fitting the SANS data was done using software modules provided by JCNS QtiKWS and NIST Igor$^{127}$ analysis packages. In order to cover the broader length scale of the network structure, very-small angle Neutron scattering (VSANS) experiments were carried out at the KWS3 diffractometer using the parabolic mirror as an optical element, and covering the smaller Q range from 0.0001 to 0.002 Å$^{-1}$. $^{92}$
6.2.4 Powder X-Ray Diffraction (XRD)

X-ray diffraction (XRD) patterns on dried composite films were collected using a PAN-alytical X’Pert X-ray powder diffractometer equipped with a Cu Kα₁ radiation source (λ = 1.5406 Å).

6.3 Results and Discussion

After the first step of magnetite mineralization, small magnetite nanoparticles formed and attached to the gel network as described in chapter 4. The next step started after reloading of ferrous ions into the formed gel-magnetite composites. The gels became much darker indicating more/larger particle formation inside. Further placing the gels in KOH and KNO₃ solutions for several minutes, strong magnetic responsive formed after few minutes to several hours.

![Graph](image)

**Figure 6.1.** SANS-VSANS scattering intensity against Q of a typical sample synthesized by a two-step method, by which 18 wt% gelatin hydrogels with FeCl₂ (0.2 M) were treated in KOH (0.1 M), KNO₃ (0.5 M) solution. The sample was placed in a 28 vol% D₂O solution. The solid line is the best fitting by the Beaucage equation.
Two step Magnetite Mineralization in Gelatin Hydrogels

The open circles in Figure 6.1 represent the scattering of the composites in a 28 vol% D$_2$O aqueous solution, which matches the gelatin scattering and visualize the magnetite nanoparticles. The scattering in the intermediate Q-range caused from individual nanoparticles of R$_g$ = 470 (±2) Å show a Q$^{-1.3}$ power law at intermediate Q, which might indicate a rod-like shape. In contrast, in Chapter 5 the large magnetite nanoparticles are suggested to have spherical shape with a fractal inner-structure. The scattering curve in the high Q shows Q$^{-4}$ scattering indicating dense particles with a smooth interface. Further, the scattering curve has a cross over Q$_c$ (~0.04 Å$^{-1}$) in between the Q$^{-1}$ and Q$^{-4}$ reflecting a small structure with the size less than 150 Å. This small structure may come from the primary particles and they may serve as the building unit as the large particles.

![Figure 6.2](image)

**Figure 6.2.** SANS-VSANS scattering intensity against Q of the sample measured at different time stages, by which 18 wt% gelatin hydrogels with FeCl$_2$ (0.2 M) were treated in KOH (0.1 M), KNO$_3$ (0.5 M) solution. The sample was placed in a 28 vol% D$_2$O solution. The inset R$_g$ plots obtained from the fitting of the Beaucage equation.

In order to further clarify the formation kinetics, Figure 6.2 shows the magnetite mineralization at different time stages. The sample at 0 min means a sample measured as the newly formed magnetite by the coprecipitation step (first step with R$_g$ ~ 59 Å). With time reaching 1 minute, the scattering intensity has a significant increase in the low Q
range. The high Q regime show a increase of the power law slope from -2 to around -4 indicating a dense particles formation. The inset picture shows $R_g$ has that is nearly constant at the ~ 60 Å very early stages while the particle grows significantly after 5 min. The scattering intensity increases with the time without size change. A possible reason may be caused by the newly formed iron hydroxide and the free Fe$^{2+}$ reacting with the primary particles thus compacting the particle inner-structure. The new reaction start may induce the particle-particle interaction strongly thus a structure factor can be used to describe the interaction. In additional, a sample in the second step mineralization without KNO$_3$ was investigated. The increasing intensity in the low Q range indicates that the particle-particle interaction appears due to the system net charge changing.

Figure 6.3. XRD patterns of magnetite-gelatin hybrid dry gels as a function of mineralization time.
Figure 6.4. SANS-VSANS scattering intensity against Q of the sample measured at different time stages, by which 18 wt% gelatin hydrogels with FeCl$_2$ (0.2 M) were treated in KOH (0.1 M), KNO$_3$ (0.25 M) solution. The sample was placed in a 28 vol% D$_2$O solution. The inset $R_g$ plot is obtained from the fitting of the Beaucage equation.

Figure 6.3 represents XRD results when a sample mineralization at 5 minutes was measured with a dry gelatin sample for comparison. The result shows typical magnetite peaks and thus the magnetite phase is conformed. However, by the two-step method, it seems that the gelatin crystal structure disappeared.

Figure 6.4 shows a lower KNO$_3$ concentration condition, the SANS was measured at different time stages. Comparing the results with Figure 6.2, the SANS results show similar trend. The most different is the final particles dimension. The intermediate Q scattering changes from Q$^{-1}$ to Q$^{-2}$ scattering indicating a rigid rod-like structure to a flexible chain-like shape (intermediate products) transition.

### 6.4 Conclusion

In this chapter, we use a two-step method combining the coprecipitation and the partial oxidation method to produce large magnetite particles. The obtained results show that dense large magnetite nanoparticles with a size of $R_g \sim 460$ Å formed into two shapes of particles, rigid rod-like and flexible chain-like particles, which were controlled by the KNO$_3$ concentration (see Figure 6.5). The time-dependent experiments show that the
primary particles were compacted by interaction with the early stage iron precipitate and iron ions in the early stage of the mineralization.

Figure 6.5 Summary and schematic representation of the results of two-step magnetite nanoparticles formation in the presence of gelatin hydrogels in D$_2$O/H$_2$O 28 V/V% obtained by SANS/VSANS.
7 Magnetite Mineralization in Gelatin Hydrogels in Nacre Organic Matrix

7.1 Introduction

Biominerals are inorganic/organic hybrid composites formed under conditions of moderate temperature, pressure and pH showing remarkable materials properties and controlled hierarchical structures, which has received much attention because they are considered as natural archetypes for future materials.\textsuperscript{17-24} It is known that in the composites the small amounts of organic component are essential for the formation of biominerals.\textsuperscript{25} These biomacromolecules not only significantly improve the mechanical properties of the resulting composites but also exert a crucial control on the biomineralization process, contributing to the determination of the crystal size, polymorph, morphology and crystallographic orientation.\textsuperscript{1,26} Therefore, biological routes of structuring biominerals are becoming valuable approaches for practical engineering processes.

An intriguing and much-investigated material is nacre. Known as the mother of pearl, nacre is the iridescent inner surface of some molluscan shells, which consists of highly oriented aragonitic crystals and an organic matrix (polysaccharides and proteins).\textsuperscript{39,40} It is well known for its beautiful iridescence but also for the outstanding mechanical properties e.g. high fracture toughness. This organic/inorganic hybrid structure makes nacre 3000 times more fracture resistant as compared to aragonite which makes up ca. 95 wt.-% of this structure.\textsuperscript{41}

Another amazing example of biomineral is the teeth of chitons which consists of a magnetite/protein-polysaccharide hybrid shell.\textsuperscript{52} The fully mineralized chiton teeth display remarkable functional properties such as outstanding fracture toughness, wear resistance and the highest reported hardness among known biominerals.\textsuperscript{48,168} Proteins and a pre-formed polysaccharide fibrous hydrated network are buried within the teeth and provide a spatially confined template for the growth of a tooth's magnetite shell of defined geometry.\textsuperscript{49} It is quite evident that the interaction between the inorganic mineral and the organic matrix interface\textsuperscript{169} is essential for an understanding of the whole process, which
Magnetite Mineralization in Gelatin Hydrogels in Nacre Organic Matrix

may open new ways of new material design strategies and the generation of materials with improved chemical and physical properties.

We thus attempted to explore, inspired by nature’s fabrication strategies, the use of biomolecules in the study of mineralization of a material combining the properties of nacre and Chiton tooth material, particularly shedding light on the mechanistic aspects of the processes control. In detail, the insoluble organic nacre matrix of the shell *haliotis laevigata* was used as a special environment for the ion diffusion and mineralization. Within the nacre organic matrix gelatin was infiltrated to mimic the gel precursors inside the chitin nacre scaffold. Inside this organic gelatin matrix magnetite was produced to form a highly mineralized organic-inorganic hybrid body.

The following presented data was performed in collaboration with different groups in a Priority program project of the DFG (SPP 1569 Generation of multifunctional inorganic materials by molecular bionics). Samples synthesis and parts of non-scattering characterization are closely collaborated with Maria Siglreitmeier (a Ph.D. student in AG Cölfen, Uni Konstanz). Parts of the presented results in this chapter were adapted from the following publications:


### 7.2 Experimental Section

#### 7.2.1 Materials

Materials: The following commercially available chemicals were purchased and applied in the synthesis without further purification: FeCl₂·4H₂O (Sigma-Aldrich), FeCl₃·6H₂O (Sigma-Aldrich), NaOH (Sigma-Aldrich), D₂O (99.8% D) (ARMAR Chemicals), Gelatin Type B (~225 Bloom, Sigma-Aldrich), 4-chloro-m-cresol (Fluka), Methanol (VWR). Acetic acid (Sigma-Aldrich), EDTA (Sigma-Aldrich). The shells of *haliotis laevigata* come from Australia. For the preparation of the reactant solutions double-
distilled and deionized (Milli-Q) water was used. All iron including solutions were
degassed with argon before use.

7.2.2 Preparation of insoluble organic nacre matrix
Shells of *haleotis laevigata* were sandblasted to remove the calcite layer. After thorough
washing with deionized water, the shells were dried overnight at room temperature and
cut into rectangular pieces with a length of around 1 cm x 1 cm. The nacre pieces were
demineralized with 2-10 vol % acetic acid or 200 Mm EDTA and solvent exchange eve-
ry day for at least 5 d at 6 °C (Figure 7.1). The remaining organic matrix was washed
with Milli-Q water until neutral pH was reached.

![Figure 7.1. Schematic representation of experimental steps. (1) Nacre demineralization. (2) Infiltration with gelatin. (3) Magnetite Mineralization in the hybrid organic matrices.](image)

7.2.3 Infiltration of gelatin inside the insoluble nacre matrix
Different amounts of gelatin were allowed to swell in water for 24 hours at 6 °C. Ho-
mogeneous solutions were prepared by heating these gels for 2 hours at 50 °C. In each
case, 20 ml of the solution is filled in crystallization dishes and allowed to form a gel
there. To avoid decomposition by bacteria, a 5 wt.-% solution of 4-chloro-m-chresol in
methanol was added (0.15 ml per 1g of dry gelatin).
The cut demineralized insoluble organic nacre pieces were placed into crystallization dishes filled with 20 mL liquid gelatin at 55°C (Figure 7.1). To confirm uniform contact of the matrix pieces with the melted gelatin sol solution a filter paper covered the liquid surface to prevent floating. The complete set-up was then placed into a vacuum desiccator and the desiccator was attached to a vacuum pump. The vacuum was then applied until bubbling of the solution was observed. The vacuum was then removed to force the liquid gelatin to be drawn inside the tissue. The whole process was repeated three times. After gelatin infiltration, the nacre matrix pieces were left inside the gelatin filled crystallization dishes and allowed to stand for gelation first 5 h at room temperature and finally kept at 6°C for 24 h before further use. For further procedure the gelatin filled insoluble organic nacre parts were cut out of the gelatin hydrogel with a scalpel.

7.2.4 Magnetite Formation in Gelatin-Nacre Organic Hybrid Matrix

In situ mineralization of magnetite nanoparticles in the presence of gelatin/nacre organic hybrid matrix was carried out via co-precipitation of FeCl$_2$ and FeCl$_3$ after an already established synthesis protocol mentioned in the Chapter IV (Figure 7.1). Briefly, the gelatin/nacre organic hybrid matrix samples were introduced into a solution, containing FeCl$_2$ (0.1 M) and FeCl$_3$ (0.2 M), and left for 96 hours at around 5°C. The iron (II) and iron (III)-loaded matrix was washed with water and placed into 0.1 M NaOH solution for 150 min.

7.2.5 SANS and VSANS Experiments

Small-Angle Neutron Scattering (SANS) and Very-Small Angle Neutron Scattering (VSANS) experiments were carried out at, respectively, the KWS1 and KWS3 diffractometers of JCNS outstation at Heinz Maier-Leibnitz Zentrum (MLZ) in Garching, Germany$^{92}$. Four configurations were used at KWS1, namely the sample-to-detector (SD) distances of 1.3, 2, 8 and 20 m, the corresponding collimation length of 8 and 20 m, and a wavelength of 7 Å ($\Delta \lambda / \lambda = 10\%$). These settings allowed covering a Q-range from 0.002 to 0.35 Å$^{-1}$. The scattering vector Q is defined as $Q = 4\pi \lambda \sin\theta$ with the scattering angle $\theta$ and the wavelength $\lambda$. A two-dimensional position sensitive detector was used to detect neutrons scattered from sample solutions.
Thin film samples characterized by SANS and VSANS were hydrated in D$_2$O (or a mixture of D$_2$O/H$_2$O) and fixed in a sandwich quartz cell with a path length of about 1 mm (Figure 7.2).

![Diagram](image)

**Figure 7.2.** (a) The nacre tablet structure and (b) SANS scattering geometry on the nacre and nacre organic matrix.

Some of the SANS data were measured at SANS II at Paul Scherrer Institute (PSI) in Villigen, Switzerland. The sample-to-detector distances were 1 and 5 m, the corresponding collimation lengths 4 and 5 m, and the wavelength 5.2 Å. Water was used as a secondary standard to calibrate the scattering intensity in absolute units. These settings allowed covering a Q-range from 0.01 to 0.35 Å$^{-1}$.

The data correction and calibration were performed using the software QtiKWS$^{96}$. In order to cover a broader length scale of the network structure, very-small angle Neutron scattering (VSANS) experiments were carried out at the KWS3 diffractometer using the parabolic mirror as an optical element, and covering the smaller Q range from 0.0001 to 0.002 Å$^{-1}$ $^{92}$.

Fitting the SANS data was done using software modules provided by JCNS QtiKWS$^{96}$ and NIST Igor$^{127}$ analysis packages. Some expression and models for the hybrid materials as following:

**The Beaucage Expression**
The Beaucage empirical expressions are able to reasonably approximate the scattering from many different types of particles, including fractal clusters, random coils (Debye equation), ellipsoidal particles, etc.\textsuperscript{103} The Beaucage expression is given according to:

\[
\frac{d\Sigma}{d\Omega}(Q) = \frac{d\Sigma}{d\Omega}(0) \exp(-u^2/3) + P_a [(\text{erf}(u/\sqrt{6}))^3 / Q]^\alpha
\]

representing a combination of Guinier’s and Porod’s laws describing the scattering at low and large \( Q \), respectively. More detail can see in chapter 2.

In this chapter, the scattering from the inorganic minerals and the nacre structure are fitted with Beaucage expression to generate the size and dimensions of a sample.

**The Correlation Length Model**

The correlation length model developed by B. Hammouda\textsuperscript{104} is made of two terms. The first term describes Porod scattering from clusters (exponent = \( n \)) at small \( Q \) and the second term is a Lorentzian function describing scattering from polymer chains (exponent = \( m \)) at larger \( Q \). The second term \( C/[1+(Q\xi)^m] \) characterizes the polymer/solvent interactions and therefore the thermodynamics. The two multiplicative factors \( A \) and \( C \), the incoherent background \( BKG \) and the two exponents \( n \) and \( m \) are used as fitting parameters.\textsuperscript{79, 104}

\[
\frac{d\Sigma}{d\Omega}(Q) = \frac{A}{Q^n} + \frac{C}{1+(Q\xi)^m} + BKG
\]  

(7.2)

In this chapter, the composites in \( \text{D}_2\text{O} \) corresponding to the organic matrix structures are fitted with correlation length model to generate the correlation length and to evaluate the clustering strength.

**7.2.6 SAXS Experiments**

SAXS experiments were carried out at a HECUS S3-Micro and GALAXI\textsuperscript{97} small-angle X-ray scattering instrument. The HECUS S3-Micro instrument uses Cu K\( \alpha \) radiation (0.154 nm) produced in a sealed tube. The GALAXI instrument uses Metaljet source (\( \lambda = 1.34 \) Å). Gel samples were placed in Hilgenberg quartz capillaries with an outside diameter of 1 mm and wall thickness of 0.01 mm. The scattered intensity was corrected with the transmission of the samples calculated considering the absorption of the sample and that of the capillary. The nacre organic matrix samples were dried and measured.
directly. The scattered X-rays are detected with a two-dimensional multi-wire area detector and afterwards converted to one-dimensional scattering by radial averaging and represented as a function of momentum transfer vector $Q$ similar to the SANS experiments.

### 7.2.7 Transmission Electron Microscopy (TEM)

For TEM analysis, a Zeiss Libra 120 operating at 120 keV and a JEOL JEM-2200FS operating at 100 keV were used, respectively. For material characterization, a drop of a diluted dispersion of nanoparticles extracted from the hydrogel was placed on a formvar carbon coated copper grid and left to dry on a filter paper for measurements.

### 7.2.8 Scanning Electron Microscopy (SEM)

For SEM analysis, a Zeiss Neon 40 EsB operating in high vacuum was used. An InLens and SE detector were used for signal collection and an acceleration voltage of 2 kV was chosen for recording the images. The specimens were coated with a thin layer of gold in order to avoid charging effects.

### 7.3 Results and Discussion

#### 7.3.1 The Hierarchical Structure of Nacre Organic Matrix

Nacre, an inorganic and organic composite natural material, is typically found on the mollusks inner shells and is referred to as mother-of-pearl. It is build up through a layered arrangement of pseudo-hexagonally shaped aragonite mesocrystals with a diameter of around 10 – 15 μm and a thickness of about 500 nm \(^47\) (every platelet consisting of 10 – 45 nm polygonal CaCO\(_3\) nanograins\(^195\)). The aragonite tablets are interspaced by an organic matrix which was identified as a β-chitin\(^40, 196, 197\) core surrounded by protein layers playing an important role in the nacre formation process\(^198-200\). Mineral bridges connect the aragonite tablet. Their width ranges from 36 - 54 nm in between the neighbor lamellar which represent the continuation of mineral growth along the vertical direction of the lamellae platelets from a preceding layer of platelets\(^201\). The fraction of the organic matrix in nacre is only ~5wt.-%, it plays an important role in spatial control of mineralization, hierarchical structure and toughness enhancement \(^40, 43\). Although the organic matrix has been characterized both chemically and structurally, we are still far
from fully understanding the matrix structure. We, therefore, examined the structure of the nacreous organic matrix by the non-destructive method of small-angle neutron scattering (SANS) that avoids staining and dehydration. The structure of the original nacre layer was also investigated for comparison.

Figure 7.3. SANS macroscopic cross-section dΣ/dΩ versus scattering vector Q for a 1 mm thick piece of nacre in air and a demineralized nacre matrix in D₂O (T = 20 °C). The neutron beam is parallel to the nacre/nacre organic matrix c-axis (perpendicular to the sample surface). At low Q (<0.002 Å⁻¹) VSANS data are also presented after rescaling. The solid line represents a fit of the Beaucage equation and correlation length model (Q > 0.003 Å⁻¹). The insets are 2D scattering of the VSANS large structure.

Figure 7.3 represents SANS profiles of nacre (top) and its organic matrix (bottom) measured at two diffractometers for very small (VSANS) and conventional small angular scattering (SANS) in, respectively, Q-ranges from 10⁻⁴ to 2·10⁻¹ Å⁻¹ and from 10⁻³ to 0.35 Å⁻¹. The absolute value of the scattering vector Q is related to the scattering angle 2θ and neutron wavelength λ according to Q = (4π/λ)sinθ. The neutron beam is parallel to the nacre/nacre organic matrix c-axis (perpendicular to the sample surface, see Figure 7.2). Thus nearly no information on the thickness of the lamellar platelets is found in the scattering curves. These measurements enable the determination of the hierarchical structures along the vertical direction of the lamellar platelets of the nacre and its organic matrix over a wide range from microscopic to macroscopic length scale, i.e.
from about 1 nm to 1 μm. The data in Figure 7.3 show several distinct Q-regimes which are well described by the solid line representing the best fit of the data using Beaucage’s expression and a correlation model. For nacre, scattering from the aragonite tablets is dominant in the Q regime less than 0.002 Å⁻¹ and is represented by a Q⁻² power law with an amplitude of P₂ = 180 cm⁻¹ Å⁻². This exponent implies a platelet-like structure characteristic with a plate diameter larger than 2 μm as evaluated from Rg, assuming the form factor of a thin plate-like shape. Above Q* ≃ 0.0063 Å⁻¹ the power law transforms into Q⁻³ and above 0.04 Å⁻¹ to a Q⁻⁴ Porod behavior, delivering an average size of the nanograins of about 100 Å as estimated from D ≃ 2πQ². The diameter of the nanograins is around 110 Å as evaluated from Rg, assuming the form factor of a spherical shape, which is consistent with data reported in the literature. The scattering following the Q⁻² power law between 3×10⁻³ and 0.02 Å⁻¹ shows the presence of a shoulder which might correspond to the mineral bridges of average diameter estimated roughly as D ≃ 800 Å from Rg ≃ 289 Å.

The scattering profile of the nacre organic matrix (lower Figure 7.3) indicates the same platelet-like structure as for nacre as it shows the same power laws, however with an order of magnitude smaller amplitude of P₂ = 13 cm⁻¹ Å⁻². This means that demineralization has no significant influence on the original structure of the organic matrix. Above Q = 0.003 Å⁻¹ a radius of gyration Rg of about 243 Å is determined which might correspond to the holes which are the former mineral bridges in the biominerals. The diameters of the holes cross section were roughly estimated as D ~ 680 Å from the Rg ~ 243 Å, this result is consistent with our TEM results. The size of the hole is much larger than the typical size of the gelatin molecule of Rg ~ 64 Å (at 50°C) with SAXS (see chapter 3, Figure 3.20 C), indicating possible gelatin molecular diffusion into the organic matrix through the holes. Above Q = 0.05 Å⁻¹ scattering from around 0.08 Å large species appears, representing the chitin chain scattering. In addition, the inset 2D low Q scattering images show anisotropy due to the aragonite tablet orientation. The nacre organic matrix sample has much clear hexagonal scattering than nacre by VSANS due to the higher contrast after removing the minerals.

In summary, we can conclude that nacre is completely demineralized by our experimental procedure and that the structure of the demineralized nacre organic matrix has not significantly changed compared with the original nacre.
In Figure 7.4 a result from SAXS on a dry nacre organic matrix sample is presented. The scattering curve can be divided into two parts. The scattering below 0.005 Å⁻¹ follows a power law Q⁻³ scattering indicating a mass fractal structure (fractal of the open large pores), which was induced by the drying effect. The scattering above 0.005 Å⁻¹ shows Q⁻¹ scattering due to the chitin molecules. The data was fit with a fractal-cylinder model¹³⁶,¹³⁷ which describes the system similar to the dry chitin matrix. A chitin structure is presented left in the inset picture. The fitting results indicate cylinders with a radius of ~5.1 Å and length of ~123 Å which are correlated with an average distance of ~ 70 Å (the mesh size). WAXS result shows a peak with the d-space value of 4.5 Å indicating a partly order chitin molecular.

![Figure 7.4. SAXS scattered intensity versus scattering vector Q for dry Nacre organic matrix. The solid line represents a fractal cylinder model¹³⁶,¹³⁷ fitting. On the top-right is a WAXS result and a schematic representation of the dry matrix structure (left).](image)

In Figure 7.5 is the typical hierarchical structure of nacre and nacre organic matrix. The tablet thus has a thickness of ~0.25 to 0.5 µm and few µm in size. The organic matrix in Figure 7.5 d shows no collapse and with hole-size of ~650 Å which is consistent with the SANS-VSANS results.
Figure 7.5. The hierarchical structure of nacre and nacre organic matrix. (a) Shells of *Haleotis laevigata*. (b) SEM image of the nacre platelet structure. (c) A cartoon of nacre platelet structures (on top view). (d) A fresh nacre organic matrix piece. (e) TEM result on the chitin sheet structure. (f) A cartoon of nacre platelet structures and chitin layer (on the side view). (g) α-chitin texture. (h) Chemical structure of chitin.

Figure 7.6. SANS macroscopic cross-section dΣ/dΩ versus scattering vector Q for (1) nacre powders, (2) 1 mm thick piece of nacre in air and a demineralized nacre matrix in D₂O (T = 20°C) by (3) strong acetic
acid and (4) EDTA. The neutron beam is parallel to the nacre/nacre organic matrix c-axis (perpendicular to the sample surface). At low Q (< 0.002 Å⁻¹) VSANS data are also presented after rescaling.

Scattering results indicate a multiple level structure which corresponds to the nacre tablet structure, the mineral bridges, and the mineral nanoparticles. The structure is sensitive to the sample preparation and status. SANS is an important non-destructive technique which allows to measure the sample in an original state that we can evaluate the sample sensitive structure in-situ. Figure 7.6 displays SANS results on original nacre and the powders. The scattering over a Q value of 0.04 Å⁻¹ shows similar scattering indicating the same mineral structure. However, the scattering below 0.04 Å⁻¹ reveals large difference that the nacre tablet and mineral bridge structure are broken after the nacre was grounded to powders. The nacre organic matrix obtained from strong acetic acid demineralization resulting the tablet structure collapse. These results were found by examining the power law slope transition from -2 to -3 in the low Q scattering.

### 7.3.2 Gelatin Hydrogel inside of Nacre Organic Matrix

![Figure 7.7. VSANS scattering patterns of nacre organic matrix and gelatin-nacre organic matrix hybrids in D₂O.](image)

After inducing gelatin in a nacre organic matrix, one important point is the status of the gelatin. Does the gelatin molecule interacted with the nacre organic matrix or do they stay outside of the nacre matrix? Is there any collapse of the nacre organic matrix? The gelatin position in nacre can be easily confirmed by light microscopy where the dye
labeled gelatin can be found in between a layered structure.202 Figure 7.7 shows the VSANS results on the gelatin-nacre organic matrix hybrid structure. In the Q range the scattering reflects the nacre tablet structure. Comparing with pure gelatin scattering, the low Q scattering in the hybrid scattering are dominated by the nacre organic matrix. Both samples have a $Q^{-2}$ scattering after including the gelatin into the nacre structure. There is no strong crosslink or collapse of the nacre organic matrix. The gelatin should follow the structure of the nacre tablet which is not changed during the gelatin addition.

7.3.3 Magnetite formation in the Hybrid Organic Matrix

![Figure 7.7](image)

**Figure 7.8.** SANS and VSANS scattering patterns of magnetite in a gelatin-chitin composite and of a ferrogel in a mixed D$_2$O/H$_2$O solvent of 28 vol% D$_2$O and 72 vol% H$_2$O. The solid lines represent the fitting of the Beaucage expression (equation 7.1).

In order to further clarify the magnetite-gelatin-chitin structure of the hybrid material, we performed SANS contrast variation experiments which allow to independently explore the inorganic nanoparticle structure as well as the organic network. Contrast variation SANS experiments became a standard tool as pointed out and applied in cognate disciplines such as biomineralization$^{175, 176}$. Figure 7.8 displays two SANS – VSANS scattering patterns of magnetite in a chitin-gelatin composite (top) and in a gel-
Magnetite Mineralization in Gelatin Hydrogels in Nacre Organic Matrix

atin matrix (bottom) dissolved in an aqueous mixture of 28 vol% D$_2$O matching the scattering of the organic components. The structure of the ferrogel (the hybrid material without chitin) was investigated for comparison. The magnetite-gelatin-chitin sample shows a power law of $Q^{-1}$ in the low Q-regime (< 0.0001 Å$^{-1}$), approximately valid for linear structures thereby indicating rod-like particles or chains of particles of about $R_g = 0.58 \, \mu m$. At larger Q ($> 0.01 \, \text{Å}^{-1}$) scattering is determined from individual magnetite nanoparticles of $R_g \approx 79 \, \text{Å}$ showing a $Q^3$ power law indicating a mass fractal structure (a structure containing branching and crosslinking to form a 3D network). The diameter $D$ of the magnetite particles can be estimated as $D \approx 200 \, \text{Å}$ ($D = 2\sqrt{5/3}R_g$) assuming a spherical shape. The scattering of magnetite in the gelatin matrix (ferrogel) looks qualitatively the same. Particles (or an assembly of particles) of about $R_g = 0.6 \, \mu m$ with $Q^2$ power law, characteristic for chain-like clusters are found at small Q. Individual magnetite particles become visible at larger Q showing a slightly smaller diameter of about $D \approx 185 \, \text{Å}$ ($R_g = 72 \, \text{Å}$). Thus, in the presence of nacre organic matrix, the chitin fiber like structure helps the formation of linear aligned magnetite nanoparticles (pearl necklace like, power law of $Q^{-1}$), while in the gelatin gel matrix the nanoparticles follow a branch-like arrangement (power law of $Q^{-2}$).

### 7.4 Conclusions

In summary, we attempted to explore, inspired by nature’s fabrication strategies, the use of biomolecules in the study of mineralization of material with the combined properties of nacre and Chiton tooth material, particularly shedding light on the mechanistic aspects of the control the processes. In detail, the insoluble organic nacre matrix of the shell *Haliotis laevigata* was used as a special environment for the ion diffusion and mineralization. Within the nacre organic matrix, gelatin was infiltrated to mimic the gel precursors inside the chitin nacre scaffold. Inside the hybrids, magnetite was produced to form a highly mineralized organic-inorganic hybrid body. The following points can be concluded:

1) SANS represents typical results from the natural organic nacre matrix representing a hierarchical structure from macroscopic to molecular length scale. The scattering results indicate four structural levels which correspond to the tablet structure (> 2 µm), the mineral bridges ($R_g \approx 243 \, \text{Å}$), the mineral nanograins ($R_g \approx 45 \, \text{Å}$) and the chitin matrix...
(radius of ~5 Å and length of 120 Å). Those structure parameters are sensitive to the sample status. It is very important, that demineralization has no significant influence on the original structure of the organic matrix.

2) The size of the hole appears larger than the typical size of the gelatin molecule as determined from the $R_g$ of 64 Å with SAXS, indicating possible gelatin molecular diffusion into the organic matrix through the holes. After inducing gelatin into the nacre organic matrix, there is no change of the nacre tablet like structure.

3) In the presence of nacre organic matrix, the chitin fiber like structure helps to linearly align magnetite nanoparticles (pearl necklace like, power law of $Q^{-1}$), while in the gelatin gel matrix the nanoparticles follow a branched arrangement (power law of $Q^{-2}$).
8 Summary and Outlook

8.1 Summary

The present thesis deals with the investigation of the mechanism of bio-inspired magnetite mineralization in gelatin hydrogels as well as the organic-inorganic hybrid structures by Small Angle Neutron / X-ray Scattering methods. The results provide important information for better understanding and prediction of bio-inspired magnetite mineralization. This will help to improve the bio-inspired organic-inorganic hybrid materials synthesis. Figure 8.1 is a summary and schematic diagram of the chapter organisation.

In chapter 3, investigation of the gelatin hydrogels using SANS and VSANS revealed the existence of two phases: one is the colloid-like large clusters filled mainly with densely packed gelatin triple-helices (“dense phase”); the other is a 3D cage-like gel network composed of gelatin triple-helices and random coils (“dilute phase”). In the “dilute phase” the ratio between gelatin triple-helices and random coils is a function of gelatin concentration. The volume fraction of the “dense phase” is proportional to the gelatin concentration. The gel structure is well described by a multi-level model with next parameters: radius of gyration of the large clusters or the gelatin triple-helices $R_g$, the gelatin three dimensional cage size $R_m$, correlation length of the short range intra and inter-chain average distance $\xi$, and the clustering strength $A/Q^n$ being a term to evaluate the aggregation strength. The gelatin structure was investigated as a function of gelatin concentration, ratios of $D_2O/H_2O$, temperature, pH, salts concentration and cation valence. Those experimental conditions influence the hydrogel properties via change of the gel structures, e.g. the triple-helix to random coil ratios in the dilute phase, the aggregation of large clusters, length and diameter of the triple helices. A detailed study of the gelatin structure helped to choose appropriate gelatin concentrations, temperature, pH to achieve high efficiency for ion transport, optimal iron mineralization as well as high mechanical strength.
Chapter 4 presented a bio-inspired mineralization of superparamagnetic magnetite nanoparticles by coprecipitation of ferrous and ferric ions using the gelatin hydrogels as the pre-formed network. By the pathway we want to mimic the magnetite mineralization in Chiton teeth. The role of gelatin hydrogels and the bio-inspired magnetite mineralization mechanism have been explored by using small angle scattering methods in conjunction with TEM/AFM/XRD measurements. The composites in $\text{D}_2\text{O}$ (mainly gelatin has a scattering contrast to solvent) and 28 vol% of $\text{D}_2\text{O}$ (mainly minerals have contrast) have been investigated in order to determine separately the roles of the organic and inorganic
components. The iron concentration, drying induced microstructure reorganization as well as the time-dependent mineralization have been intensively investigated through probing the multi level structure changes in the organic matrix and the mineral properties (shape, size, distribution). This chapter is aimed to show the correlation between the spacing confinement of the gelatin matrix and the mineralized particles. In detail, SANS shows with respect to gel concentration an unchanged gelatin network structure of average cage size larger than the magnetite nanoparticles. The mineral size of $\sim 110$ Å seems to be independent of the gelatin concentration and limited by the gel cage (the matrix in particle structure). The gelatin macromolecules influence the mineralization mechanism due to the strong interaction between the gelatin helices-coils and ferric ions. It seems that Fe$^{3+}$-rich helices of the gelatin matrix serve as nucleation centers of the co-precipitation that leads to attachment to the gelatin matrix structure.

Chapter 5 presented a bio-inspired method of formation of larger magnetite nanoparticles by partial oxidation of ferrous ions at basic conditions in the presence of the gelatin hydrogels, which closely mimics the mineralization strategy taken by magnetotactic bacteria. The size of the large nanoparticles with diameters between 300 to 1500 Å seems to be independent of the gelatin concentration and include the gelatin matrix to form a “matrix in particle” structure. SANS-VSANS results show a gelatin network structure of average 3D-cage size smaller than the magnetite nanoparticles. The Fe$^{2+}$ ions show weak interactions with the gelatin matrix resulting a homogenous-like mineralization, which makes it possible to grow large particles in the gel matrix. A possible mechanism of the large magnetite nanoparticle formation is proposed: the magnetite nanoparticles are formed via a rod-like intermediate particles formation and transition to large magnetite particles after 30 minutes. Both magnetic (magnetite) and non-magnetic phases were detected during the mineralization. One-dimensional alignment of individual large nanoparticles was detected after 1 hour; this was induced by the magnetic dipolar moment of particles in the highly swollen gelatin network.

Chapter 6 combined the two methods of magnetite mineralization to produce large particles in two steps. The obtained results show that “large” magnetite nanoparticles with a size of $R_g \sim 460$ Å are formed into two shapes of particles (particle clusters), rod-like and chain-like particles. The final shape is controlled by the KNO$_3$ concentration. The
time-dependent experiments show that in the early stage of the mineralization the primary particles became dense by interacting with the early stage iron precipitate and iron ions.

**Chapter 7** explored, inspired by nature’s fabrication strategies, the use of biomolecules in the study of mineralization of a material combining the properties of nacre and Chiton tooth material. In detail, the insoluble organic nacre matrix of the shell *Haliothys laevigata* was used as a special environment for the ion diffusion and mineralization. Within the nacre organic matrix, gelatin was infiltrated to mimic the gel precursors inside the chitin nacre scaffold. Inside the hybrids, magnetite was produced to form a highly mineralized organic-inorganic hybrid body. SAS results from the natural organic nacre matrix represents a hierarchical structure from macroscopic to molecular length scale. The results indicate four structural levels corresponding to the tablet (> 2 μm), the mineral bridges (Rg ~243 Å), the mineral nanograins (Rg ~45 Å) and the matrix (radius of ~5 Å and length of 120 Å). After infiltration of gelatin into the nacre organic matrix, there is no change on the nacre tablet like structure. In the nacre organic matrix, the chitin fibers serve as a template with the formation of linearly aligned magnetite nanoparticles.

Several key points of the mechanism of magnetite mineralization in hydrogels obtained by using small angle scattering in combination with other tools could be made **Throughout the Thesis.**

**First**, the confinement effect of the multi-level hydrogel structure limits the size of the small particles and influences the ionic diffusion during the large particle formation. **Second**, the interaction between ions and network is another key parameter in controlling the mineralization mechanism. Strong interaction between ferric ions and gelatin molecules leads to an inhomogeneous mineralization and a ‘particle in matrix’ structure, while the weak interaction between ferrous ions and gelatin matrix causes a homogenous-like mineralization and a ‘matrix in particle’ structure. **Third**, by introduction of a second organic matrix, the nacre organic matrix with chitin fibrous texture, it is possible to influence the mineral organization due to the structure spatial constraints.
8.2 Outlook

Aiming for a deeper understanding of the magnetite mineralization mechanisms in the gelatin matrix inside of the nacre tablet, some suggestions were giving for the next step work. The application of the SANS contrast variation method by H2O/D2O exchange will continue to be the important tool to analyze the structure of the individual components behavior during the mineralization process. In particular, combining SANS and neutron polarization analysis will be relevant to investigate the magnetic properties on the multiscale level.

Small-angle-scattering investigation will follow the following schedule (Figure 8.1):

- Characterization of the organic-inorganic hybrid composites by changing the scattering contrast, i.e. investigation of the individual phases (organic component, magnetite, CaCO3 and possible silica when space filling of magnetite hybrid material with a second mineral phase), the structural organic component stability during the mineralization, and the morphology of the inorganic nanoparticles.
- Study of the interaction between three cations Fe3+, Fe2+, Ca2+ or the ion clusters with gelatin/chitin. Those ionic-polymer interactions may influence the mineralization kinetics a lot.
- Study of the magnetite mineralization kinetics by SANS/SAXS influenced by Ca2+, SiO4^4- and their inorganic minerals. First work is to investigate the magnetite mineralization without any additives followed by studies of the magnetite mineralization in the gelatin matrix and in nacre insoluble matrix. Second work is to apply contrast variation method to study the magnetite mineralization in the hybrid materials (gelatin in nacre tablet). Finally, investigation of the magnetite mineralization with additives followed by studies of the magnetite mineralization.
- Characterization of the surface/interfaces after the introduction of a new component by surface scattering (e.g. GISANS/GISAXS/XRR/NRR).
- Gelatin folding drives nanoparticle reorganization in the composites (e.g. induced by solvent evaporation). Modeling of the scattering pattern and description of the interaction induced magnetite aggregation, assembly, and reorganization.
- Nuclear and magnetic scattering are two scattering contributions to better determine the structure of magnetite, e.g. their size, shape, number density, and magnetic distribution.

Furthermore, comparative studies on the structure of synthetic hybrid materials and natural minerals (e.g. nacre and chiton teeth) shall be conducted.

**Figure 8.** Scheme presentation of structural characterization of the hybrid materials using small angle scattering methods.
Appendix A: General Parameters

A.1 Radius of gyration

Table A1. Radii of gyration of some homogeneous objects.76

<table>
<thead>
<tr>
<th>Shape of geometrical bodies</th>
<th>Models</th>
<th>Radius of gyration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sphere of radius R</td>
<td><img src="image1.png" alt="Image" /></td>
<td>$R_g^2 = \frac{3}{5} R^2$</td>
</tr>
<tr>
<td>Spherical shell with radii $R_1 &gt; R_2$</td>
<td><img src="image2.png" alt="Image" /></td>
<td>$R_g^2 = \frac{3}{5} \frac{R_1^5 - R_2^5}{R_1^3 - R_2^3}$</td>
</tr>
<tr>
<td>Solid rod with length L and radius R</td>
<td><img src="image3.png" alt="Image" /></td>
<td>$R_g^2 = \frac{L^2}{12} + \frac{R^2}{2}$</td>
</tr>
<tr>
<td>Thin rod ($R \to 0$)</td>
<td><img src="image4.png" alt="Image" /></td>
<td>$R_g^2 = \frac{L^2}{12}$</td>
</tr>
<tr>
<td>Thin disk ($L \to 0$)</td>
<td><img src="image5.png" alt="Image" /></td>
<td>$R_g^2 = \frac{R^2}{2}$</td>
</tr>
<tr>
<td>Ellipsoid with semi-axes a, b and c</td>
<td><img src="image6.png" alt="Image" /></td>
<td>$R_g^2 = \frac{a^2 + b^2 + c^2}{5}$</td>
</tr>
<tr>
<td>An entropically governed polymer chain (random walk in 3D)</td>
<td><img src="image7.png" alt="Image" /></td>
<td>$R_g^2 = \frac{Na^2}{6}$</td>
</tr>
</tbody>
</table>
### A.2 Scattering length density

**Table A2.** List of common matter density and their scattering length densities in the thesis.

<table>
<thead>
<tr>
<th></th>
<th>H₂O</th>
<th>Gelatin</th>
<th>D₂O</th>
<th>Maghemite</th>
<th>Magnetite</th>
<th>Hematite</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>H₂O</strong></td>
<td>H₂O</td>
<td>Type B</td>
<td>D₂O</td>
<td>γ-Fe₂O₃</td>
<td>Fe₃O₄</td>
<td>α-Fe₂O₃</td>
</tr>
<tr>
<td><strong>Density (g/cm³)</strong></td>
<td>1.0</td>
<td>1.0-1.45</td>
<td>1.11</td>
<td>4.86</td>
<td>5.15</td>
<td>5.26</td>
</tr>
<tr>
<td><strong>Neutron SLD(10⁻⁶Å⁻²)</strong></td>
<td>-0.56</td>
<td>*3.20²D, 2.10₂O</td>
<td>6.40</td>
<td>6.65</td>
<td>6.91</td>
<td>7.26</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Nacre insoluble matrix</th>
<th>Ferrihydrite</th>
<th>D-Ferrihydrite</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chitin(C₈H₁₃O₅N)₅+protein</td>
<td>FeOOH</td>
<td>FeOOD</td>
<td></td>
</tr>
<tr>
<td><strong>Density (g/cm³)</strong></td>
<td>~1.38 for chitin**</td>
<td>3.52</td>
<td>3.55</td>
</tr>
<tr>
<td><strong>Neutron SLD(10⁻⁶Å⁻²)</strong></td>
<td>~1.7 for chitin**</td>
<td>4.13</td>
<td>6.60</td>
</tr>
</tbody>
</table>

*gelatin rapidly exchanges protons with deuterons in the solvent, scattering length density adjusts according to the D₂O content of the solvent.¹⁵⁰

** The chitin SLD obtained from G Evmenenko’s work.¹³²

The other neutron Scattering Length Density-SLD are calculated from NIST: http://www.ncnr.nist.gov/resources/sldcalc.html
Appendix B: The effect of water and heavy water influence on the mineralization

B.1 Magnetite mineralization in H₂O and D₂O (Coprecipitation)

Figure B1.1. Synthesis of Magnetite in D₂O and H₂O by coprecipitation of Fe²⁺ and Fe³⁺ ions in a non-oxidation condition.
Figure B1.2. XRD patterns of magnetite coprecipitation at pH 11 in D$_2$O and H$_2$O conditions.

Figure B1.3. TEM results of magnetite coprecipitation at pH 11 in D$_2$O (A) and H$_2$O (B) conditions.

B.2 Magnetite formation in H$_2$O and D$_2$O in gelatin hydrogels

Figure B2.1. TEM results of magnetite coprecipitation at pH 11 in gelatin hydrogels in D$_2$O (A) and H$_2$O (B).
Appendix C: SANS-VSANS Experiments

C.1 Small angle neutron scattering diffractometer, KWS-1

The KWS-1 small-angle neutron scattering instrument operated by the Jülich Centre for Neutron Science at the research reactor FRM II of the Heinz Maier-Leibnitz Zentrum (MLZ). KWS-1 (Figure C1.1) is a high resolution SANS instrument due to its 10% wavelength selector and the intensity comparable to other high-end SANS instruments in the world. A high precision sample stage for heavy loads (hexapod, Figure C1.2) allows for grazing incidence small angle neutron scattering (GISANS). Dedicated sample environment, such as rheometers, non-magnetic pressure cells, magnets allow for studies of polymers, biological macromolecules, nanocomposites, colloids, complex fluids, surfactants, thin (magnetic) films, magnetic and domains structures. Recently, with the updating magnetic samples can be studied with the polarisation analysis including incident beam polarisation and polarisation analysis of the scattered neutrons.

Figure C 1.1. A picture of the SANS instrument KWS-1. 

- 181 -
Appendix

Figure C 1.2. Sample stage, Hexapod of KWS-1.91

Figure C 1.3. Sample position of KWS-1.91, 171

Technical Data of KWS-1, Overall performance 91, 171

- $Q = 0.0007 - 0.5 \, \text{Å}^{-1}$
- Maximal flux: $1.5 \cdot 10^8 \, \text{n cm}^{-2} \, \text{s}^{-1}$
- Typical flux: $8 \cdot 10^6 \, \text{n cm}^{-2} \, \text{s}^{-1}$ (collimation 8 m, aperture 30 x 30 mm², $\lambda = 7 \, \text{Å}$)

Velocity selector
Appendix

- Dornier, FWHM 10%, \( \lambda = 4.5 \text{ Å} - 12 \text{ Å}, 20 \text{ Å} \)

**Chopper**
- For TOF-wavelength analysis, FWHM 1%

**Polariser**
- Cavity with V-shaped supermirror, all wavelengths
- Polarisation better 90%, typical 95%

**Spin-flipper**
- Radio-Frequency spin flip probability better than 99.8%

**Active apertures**
- 2 m, 4 m, 8 m, 14 m, 20 m

**Aperture sizes**
- Rectangular 1 x 1 mm\(^2\) – 50 x 50 mm\(^2\)

**Sample aperture**
- Rectangular 1 x 1 mm\(^2\) – 50 x 50 mm\(^2\)

**Neutron lenses**
- MgF\(_2\), diameter 50 mm, curvature 20 mm
- Packs with 4, 6, 16 lenses

**Sample stage**
- Hexapod, resolution better than 0.01, 0.01mm

**Detector**
- Detection range: continuous 1.5 m – 20 m
- \(^6\)Li-Scintillator 1 mm thickness + photomultiplier
- Efficiency better than 95%
- Spatial resolution 5.3 x 5.3 mm\(^2\),
- 128 x 128 channels
- Max. countrate 0.6 MHz (\(t_{\text{dead}} = 0.64 \mu s\))

**C.2 Very small angle neutron scattering diffractometer, KWS-3**

KWS-3 is a very small angle neutron scattering (VSANS) instrument with the focusing mirror running by Jülich Centre for Neutron Science at the research reactor FRM II of the Heinz Maier-Leibnitz Zentrum (MLZ).\(^{92,94,150}\) Standard configuration of the instru-
ment with 9.5 m sample-to-detector distances allows performing scattering experiments with a wave vector transfer resolution between $10^{-4}$ and $3 \cdot 10^{-3} \text{ Å}^{-1}$, bridging a gap between Bonse-Hart and pinhole cameras. Second sample position at 1.3 m distances has extended Q-range of the instrument to $2 \cdot 10^{-2} \text{ Å}^{-1}$ and reached more than one-decade overlapping with the classical pinhole SANS instruments. The principle of this instrument is a one-to-one image of an entrance aperture onto a 2D position sensitive detector by neutron reflection from a double-focusing toroidal mirror. KWS-3 is an important instrument, which extends the accessible range of scattering angles to very small angles with a superior neutron flux when compared with a conventional pinhole instrumental setup. The length scale that can be analyzed is extended beyond 10 µm.¹⁵⁰

![Figure C 2.1. Q-range of the KWS-3 diffractometer for all available configurations (#0 to #5) is plotted. For comparison, the Q-range of a classical pine-hole SANS instrument is plotted too.¹⁵⁰](image)

**Technical data of KWS3, Overall performance**⁹⁴,¹⁵⁰

- Resolution:
  - $\delta Q = 10^{-4} \text{ Å}^{-1}$ (extension to $4 \cdot 10^{-5} \text{ Å}^{-1}$ possible)
Appendix

- Q-range:
  - $1.0 \cdot 10^{-4} - 3 \cdot 10^{-3} \text{ Å}^{-1}$ at 9.5 m distance
  - $1.5 \cdot 10^{-3} - 2 \cdot 10^{-2} \text{ Å}^{-1}$ at 1.3 m distance
- Neutron flux:
  - high-resolution mode: $> 10000 \text{ n s}^{-1}$
  - high-intensity mode: $> 60000 \text{ n s}^{-1}$

**Monochromator**
- MgLi velocity selector
- Wavelength spread $\Delta \lambda / \lambda = 0.2$
- Wavelength range $\lambda = 10 - 30 \text{ Å}$ (maximal flux at 12.8 Å)

**Aperture size (focus)**
- $1 \times 1 \text{ mm}^2 - 5 \times 5 \text{ mm}^2$

**Beam size at 9.5 m**
- $0 \times 0 \text{ mm}^2 - 100 \times 25 \text{ mm}^2$

**Beam size at 1.3 m:**
- $0 \times 0 \text{ mm}^2 - 15 \times 10 \text{ mm}^2$
C.3 Sample Environment

Sample cells

**Figure C 3.1.** Hellma 404-QX cells, with path length of 0.5, 1, 2 and 5mm.

**Figure C 3.2.** Sandwich cells, with path length of 0.5, 1, 2 and 5mm.
Appendix D: SAXS Experiments

Figure D 1.1. Sample cells for SAXS experiments.
Appendix E: Lists of Equations

1. The incident photon/neutron flux per unit area per second \( I_0 \) is scattered by a sample and the scattered photons/neutrons are acquired by each detector element subtending a solid angle \( \Delta\Omega \) with a detector efficiency \( \varepsilon \). The measured scattered intensity \( I_s \) is given by\(^75, 82\):

\[
I_s = I_0 \varepsilon T_s \Delta\Omega A_s d \frac{d\Sigma}{d\Omega}
\]  

(2.1)

where \( T_s \) is the sample transmission, \( A_s \) is the cross section of the beam, \( d \) is the sample thickness and \( d\Sigma/d\Omega \) is the differential scattering cross section per unit volume.

2. The scattering length density (SLD) as a function of position in the sample was defined as\(^71, 75, 79, 83\):

\[
\rho(r) = \frac{\sum_i^n b_i}{\bar{V}} (r)
\]  

(2.2)

where \( \rho \) is the scattering length density, \( b_i \) is the scattering length of the relevant atom and \( \bar{V} \) is the volume containing the \( n \) atoms.

3. Try to connect the materials properties to the atomic properties, we can make the replacement of the sum in\(^72, 75, 83\)

\[
\frac{d\sigma}{d\Omega} (Q) = \frac{1}{N} \left| \sum_i^N b_i e^{iQ \cdot r} \right|^2
\]  

(2.3)

4. By the integral of the scattering length density distribution across the whole sample and normalize by the sample volume\(^72, 75, 83\)

\[
\frac{d\Sigma}{d\Omega} (Q) = \frac{N}{V} \frac{d\sigma}{d\Omega} (Q) = \frac{1}{V} \left| \int_V \rho(r) e^{iQ \cdot r} dr \right|^2
\]  

(2.4)

5. Taking the equation 2.4 and breaking the total volume into two sub-volumes, \( V_1 \) and \( V_2 \). So at non-zero \( Q \) values
where scattering on a two-phase system the \( \frac{d\Sigma}{d\Omega}(Q) \) is proportional to the contrast.\textsuperscript{72, 75, 78, 83}

6. By using plexiglass as a secondary standard for calibration, the scattering intensity \( I_{\text{plexiglass}} \) and the measured intensity of the sample \( I_s \) could be rewritten in terms of the scattering cross-section:

\[
\frac{I_s}{I_{\text{plexiglass}}} = \frac{I_0}{T_0} \cdot \frac{T_s}{T_{\text{plexiglass}}} \cdot \frac{\Delta \Omega_s}{\Delta \Omega_{\text{plexiglass}}} \cdot \frac{A_s}{A_{\text{plexiglass}}} \cdot \frac{d_s}{d_{\text{plexiglass}}} \cdot \frac{d\Sigma}{d\Omega}(Q)_{\text{plexiglass}}
\]

The definition of solid angle is \( \Delta \Omega \approx 1/L^2 \) with the sample-detector distance \( L \). The macroscopic scattering cross section of the plexiglass measurement is \( Q \) independent. Thus, the scattering cross-section of the sample follows the next equation

\[
\frac{d\Sigma}{d\Omega}(Q)_s = \frac{T_{\text{plexiglass}}}{T_s} \cdot \frac{d_{\text{plexiglass}}}{d_s} \cdot \frac{L^2}{L^2_{\text{plexiglass}}} \cdot \frac{I_s}{I_{\text{plexiglass}}} \cdot \frac{d\Sigma}{d\Omega}(Q)_{\text{plexiglass}}
\]

7. Taking account the measurements of the empty cell scattering \( I_{\text{cell}} \), blocked beam scattering \( I_{BG} \), the final normalized scattering cross section becomes

\[
\frac{d\Sigma}{d\Omega}(Q) = \frac{I_s-I_{BG}}{I_{p\text{lexi}}-I_{BG}} \cdot \frac{T_{\text{plexi}}}{T_{EB}} \cdot \frac{d_{\text{plexi}}}{d_s} \cdot \frac{I^2_s}{I^2_{\text{plexi}}} \cdot \frac{d\Sigma}{d\Omega}(Q)_{\text{plexi}}
\]

8. SANS scattering cross section of a sample is:

\[
\frac{d\Sigma}{d\Omega}(Q) = \frac{I(Q)_s - I(Q)_b}{t_s T_s - t_b T_b} \cdot 0.0164 \cdot \frac{I}{I_{EB}} \cdot \frac{I}{I_{EC}} \cdot \frac{1}{t_w T_w} \cdot \frac{1}{t_b T_b} \cdot \frac{1}{T_{EC} T_{EC}} \]
Where $I(Q)_s$, $I(Q)_b$, $I(Q)_w$, and $I(Q)_{EC}$ are the measured intensity of sample, solution background, water and empty capillary. The bottom term in the high Q range is a flat scattering (Q independent). The T are the transmission and the t are the irradiation time.

9. In the case of a two-phase system, the scattering invariant $Q_i$ is proportional to the volume fractions $\phi_1=\phi$ and $\phi_2=1-\phi$ of phase 1 and 2 and the contrast:

$$Q_i = \int d\Omega \frac{d\Sigma}{d\Omega}(Q)dQ = (2\pi)^3 \phi_1(1-\phi_1)(\rho_2 - \rho_1)^2$$ (2.10)

10. At the Porod region, one can approximate:

$$\frac{d\Sigma}{d\Omega}(Q) \propto Q^{-n}$$ (2.11)

11. A Porod slope $n = 4$ is obtained for scattering from particles with smooth surface (sharp boundaries, as shown in figure 2.7) and thus the scattering at large Q values solely depends on the total interface area $69, 70, 72$:

$$\lim_{Q \to \infty} d\Sigma(Q) = \frac{2\pi(\Delta\rho)^2}{Q^4} \cdot \frac{S}{V}$$ (2.12)

where $S/V$ is the specific surface of the sample.

12. The Guinier approximation is formulated as $69-72, 75, 76$

$$\frac{d\Sigma}{d\Omega}(Q) \propto \frac{d\Sigma}{d\Omega}(0)e^{-(QR_g)^2/3}$$ (2.13)

or

$$\ln\left(\frac{d\Sigma}{d\Omega}(Q)\right) = \ln\left(\frac{d\Sigma}{d\Omega}(0)\right) - \frac{R_g^2}{3} Q^2$$ (2.14)

where the radius of gyration, $R_g$, is the root-mean-square of the distance of all scatters from the center of gravity. For example $69, 70$, the radius of gyration of a sphere is given by

$$R_g = \sqrt[3]{\frac{2}{5}} R$$ (2.15)
Then there is $R_{\text{sphere}} \approx 2.58 \, R_g$.

13. For an ellipsoid of half axes $a$, $b$, and $c$

$$R_g = \sqrt[3]{\frac{1}{5}(a^2 + b^2 + c^2)}$$  \hspace{1cm} (2.16)

14. For a cylinder with length of $L$ and circular cross section of radius $R$,

$$\frac{R_g^2}{L^2} = \frac{L^2}{12} + \frac{R^2}{2}$$  \hspace{1cm} (2.17)

15. It is possible to describe the distribution of material in terms of a form factor, $P(Q)$, that represents the interference of photons/neutron scattered from different positions of the same object, and a structure factor, $S(Q)$, that represents the interference of photons/neutron scattered from different objects. Then the measured intensity (corrected for background and put on an absolute scale) can be expressed as

$$\frac{d\Sigma}{d\Omega} (Q) = \frac{N}{V} (\Delta \rho)^2 \frac{2}{V} P(Q) S(Q)$$  \hspace{1cm} (2.18)

16. The form factor $P(Q)$ is the square of the complex wave amplitude $F(Q)$.

$$P(Q) = |F(Q)|^2$$  \hspace{1cm} (2.19)

17. In a none interacting systems (e.g. particles in a dilute solution), there is structure factor $S(q)=1$. In a solution, the structure factor is given by$^{72,75}$

$$S(Q) = 1 + 4\pi N_p \int_0^\infty (g(r)-1) \frac{\sin(Qr)}{Qr} r^2 \, dr$$  \hspace{1cm} (2.20)

where $g(r)$ is the pair correlation function for the scattering objects and $\ln(g(r))$ is directly related to the potential energy function that describes the inter-particle interaction.$^{69,72,75,83}$

18. For a monodisperse spherical particle with radius $r$, the form factor described as below$^{69}$

$$P(Q) = \left[ \frac{3(\sin(Qr)-Qr \cos(Qr))}{(Qr)^3} \right]^2$$  \hspace{1cm} (2.21)

19. For a monodisperse rod particle with radius $r$ and length $L = 2H$ ($L >> r$), then
where $J_1(x)$ is the first order Bessel function. Here $\alpha$ is defined as the angle between the cylinder axis and the scattering vector, $Q$.

20. The Beaucage expression is given according to:

$$
\frac{d\Sigma}{d\Omega}(Q) = \frac{d\Sigma}{d\Omega}(0) \exp(-u^2 / 3) + P_{r}[\text{erf}(u / \sqrt{6})]^3 / Q^\alpha
$$

(2.26) (3.1)

21. The following empirical functional model developed by B. Hammouda\textsuperscript{104} is made of two terms. The first term describes Porod scattering from clusters (exponent = $n$) at small $Q$ and the second term is a Lorentzian function describing scattering from polymer chains (exponent = $m$) at larger $Q$. The second term $C/[1+(Q\xi)^m]$ characterizes the polymer/solvent interactions and therefore the thermodynamics. The two multiplicative factors $A$ and $C$, the incoherent background $BKG$ and the two exponents $n$ and $m$ are used as fitting parameters. Note that when $m = 2$, this functional form becomes the familiar Lorentzian function.$^{79,104}$

$$
\frac{d\Sigma}{d\Omega}(Q) = \frac{A}{Q^n} + \frac{C}{1+(Q\xi)^m} + BKG
$$

(2.27)

22. The structure factor, $S(Q)$\textsubscript{fractal}, for a fractal rod-like network has previously been derived

$$
S(Q, D, \Xi, r0) = 1 + \frac{D\exp(\Gamma(D-1))\sin(D-1)\tan^{-1}(Q\Xi)}{(Q_0)^D[1+(Q\Xi)^{-2}]^{D-1/2}}
$$

(3.2)

where $\Gamma(x)$ is the gamma function, $r_0$ is the gauge of measurement and $\Xi$ is the characteristic length of the fractal object above which the weighted-average inter-distance of the object can no longer be described as fractal. The mass fractal dimension $D_f$ is the negative values of the power law exponent $n$. Then combineing the form factor and the
structure factor with the contrast, volume fraction ($\phi$), volume of the particles ($V_p$) and background (BKG) there is

$$\frac{d\Sigma}{d\Omega} (Q) = \phi V_p (\Delta \rho)^2 P(Q)_{cyl} S(Q)_{fractal} + BKG \quad (3.3)$$
List of Figures and Tables

Figure 1.1. Hierarchical structures of tough biominerals.  
(A) Lamellar micro-architectures of Nacre, mineral bridges between mineral tiles, the fibrous chitin network that forms the organic matrix.  
(B) Lobster exoskeleton showing the twisted plywood structure of the chitin and the tubules that extend from the chitin layers to the animal.  
(C) Antler bone image showing the hard outer sheath (cortical bone) surrounding the porous bone. The collagen fibrils are highly aligned in the growth direction, with nanocrystalline minerals dispersed in and around them.  
(D) Silica sponge and the intricate scaffold of spicules. Each spicule is a circumferentially layered rod: The interfaces between the layers assist in arresting crack propagation.

Figure 1.2. The hierarchical structure of nacre.  
(A) A sketch diagram showing the construction of nacre from a chitin molecule to a shell in bivalves. (B) Inside view of the shell. (C) A fractured transverse section. (D) SEM showing the morphology of mineralization of bivalves (top view). (E) Cryo-TEM image showing the homogenous texture and layered structure. (F) A sketch showing minerals growth through the mineral bridges. (H) Nonmineralized nacre layer matrix.

Figure 1.3. Representative images of the chiton radula, with a demonstration of the: (A) Chiton magnificus Deshayes, from Chile. (B) Chiton radula and magnet. (C) Light microscopy image of chiton radula. (D) Light microscopy image of the chiton tooth, the cross section. (E) EDX mapping results on a cross section of the chiton tooth. (F) AFM phase image of the tooth magnetite shell.

Figure 1.4. Representative images of Magnetotactic bacteria: (A) TEM image of a magnetotactic bacterium. Note the chain of twelve magnetite (Fe₃O₄) nanoparticles that are arranged along the long axis of the cell.  
(B) Model of the iron reaction pathway, and roles of the proteins that were so far shown to be necessary for magnetite biomineralization and chain formation.

Figure 1.5. Comparison of natural and artificial nacre.  
(a) Image showing natural nacre’s bright iridescence (scale bar 5 mm).  
(b) SEM image of a stack of mineral platelets on the fractured surface (scale bar 2 μm).  
(c) Organic inter-crystalline film that
allows for vertical crystal continuity between platelets (scale bar 500 nm). d, Artificial Nacre, exhibiting a similar coloration as in a (scale bar 5 mm). e, SEM image of the fractured surface showing 7 aligned CaCO$_3$ platelets separated by organic films. The surface graininess is comparable to natural nacre (scale bar 1 μm). f, SEM image of PVP film on calcite showing the a similar pore distribution as in c (scale bar 300 nm). g, AFM height image of the porous film (scale bar 300 nm).

**Figure 1.6.** Schematic representation of the materials synthetic concept. (A) Magnetite formation in a thin gelatin hydrogel 2D films). (B) Magnetite formation in thin gelatin hydrogels in the nacre organic matrix (Cellular Fractal Ferro Gel). (C) Magnetite formation in bulk gelatin hydrogels (the core and the surface mineralization may very different) (3D Bulk Gel).

**Figure 2.1.** Size range comparisons from micro to macro scale and the SAS applied range.

**Figure 2.10.** Form Factor of spheres of radius 100 Å.

**Figure 2.11.** Form Factor of rod of radius 30 Å and length 500 Å.

**Figure 2.12.** Form Factor of thin disks of radius 500 Å and thickness 30 Å.

**Figure 2.13.** Beaucage expression fitting of dense polymer clusters with $R_g$ of 30 Å which is made of Gaussian chains.

**Figure 2.14.** The correlation length model with a correlation length of 20 Å, Lorentzian exponent of 3 and porod exponent of 3.

**Figure 2.15.** A Small angle scattering (SAS) combined with Ultra-SAS and wide angle scattering investigated on structural information range on five-length scales. Structural features at larger length scales are observed at smaller Q. The picture of the materials is partly adapted from Edler.$^{107}$

**Figure 2.15.** A Small angle scattering (SAS) on rod-like micelles without shear (A) and shear-induced alignment along the horizontal direction (B) and perpendicular to the paper direction (C). In the middle are the 2D SAS scattering patterns corresponding to the real system. The bottom curve are the 1D data integration from the 2D data. The 2D images of sample A and C are treated with radial integration. Pixels with a certain radial
distance (Q) from the beam center are depicted by circles. The 2D image of sample B is integrated with a degree (χ, circle) at a fixed Q value.

**Figure 2.16.** SAS and 3D model reconstruction from for DNA cages. (a) SAXS experimental data (hollow circle) and model fit (solid lines). (b) The octahedral model is obtained from the SAXS data. (c) Overlay of SAXS model and cryo-TEM reconstruction in three different views.113

**Figure 2.17.** Schematic diagram illustrating the organization of SAS investigations on the magnetite mineralization in gelatin hydrogels.

**Figure 2.2.** Definition of scattering vector Q, with the scattering angle 2θ, the incoming beam ki and scattered beam ks.

**Figure 2.3.** A schematic representation of the effect of contrast variation on the measurable structure of a core-shell particle.

**Figure 2.4.** A schematic representation of the KWS-1 SANS diffractometer: (1) S-shaped neutron guide NL3b; (2) high-speed chopper ( = 1–10%); (3) polarizer changer; (4) radio-frequency spin flipper; (5) neutron guide sections (18 × 1 m); (6) MgF2 focusing lenses; (7) sample position with hexapod for heavy loading; (8) 3He analyzer with reversible polarization (to be implemented); (9) Anger-type scintillation detector.

**Figure 2.5.** A schematic representation of the KWS-3 VSANS diffractometer: (1) Neutron guide NL3a; (2) Velocity selector; (3) Entrance aperture; (4) Toroidal mirror; (5) Mirror chamber; (6) Sample positions 1 (10 m) and 2 (1 m); (7) Detector.

**Figure 2.6.** Schematic of a typical small-angle x-ray scattering setup. The optical system (monochromator/mirrors) selects the appropriate wavelength (∼1 Å) from the x-ray source and focuses the beam on the detector, usually a linear proportional counter or a charge-coupled detector. The lowest scattering angle of observation is determined not only by the focus size but also by the dimensions of the last aperture, which defines a region of high background (due to the optical system and slits).75, 81

**Figure 2.7.** Two systems A and B where the contrast and volume fraction are the same, but the distribution of matter is different. Both are 10% blue and 90% white. On the right is a representing of small-angle scattering curves on system A and B in Log-Log scale of dΣ/dΩ(Q) vs Q.
Figure 2.8. Guinier plot for apoferritin dilute solution (2 mg/ml). .........................- 31 -

Figure 2.9. Kratky plot \((d\Sigma/d\Omega(Q) \cdot Q^2 \text{ versus } Q)\) of scattering data illustrating changes in the behavior of the curve for folded (sphere), partially folded (sphere-random coil) and completely unfolded particles (random coil). For a folded particle, the integrated area under the curve determines the Porod invariant, \(Q\), and is scaled by concentration, \(c\). The data are adapted from Robert P. Rambo et al\(^{103}\). .........................................................- 32 -

Figure 3.1. Schematic illustrating the structure of gelatin. (A) A typical Molecular structure unit of gelatin. (B) The molecular structure of collagen in a space filling and (C) ball-stick model. The structure is obtained from a reconstruction of a PDB file (1CLG) which is from Chen\(^{119}\). ..........................................................- 45 -

Figure 3.10. Schematic representation of gelatin structure changes with the concentration: the larger cluster reconstruction from the scattering data in regime I by a rapid \textit{ab initio} shape determination program DAMMIF\(^{135}\) of (A) Gelatin 6 wt% in D\(_2\)O, (B) Gelatin 18 wt% in D\(_2\)O and (C) Gelatin 30 wt% in D\(_2\)O. Models of high Q small structure of gelatin molecules conformation changes from (D) Gelatin random coils to (E) Partly folded gelatin triple helices, and then (F) Gelatin triple-helices. .............- 62 -

Figure 3.11. SANS scattered intensity versus scattering vector \(Q\) for gelatin dry films. The solid line represents a power law fitting. The inset is an AFM result on a dry gel and a schematic representation of the gelatin structure. .................................................................- 64 -

Figure 3.12. SANS scattered intensity versus scattering vector \(Q\) for gelatin solutions from 0.1 wt% to 1 wt% in acetate-acetic acid buffer in D\(_2\)O, 100 mM, pD = 5.3. The solid lines represent the best fitting by equation 3.4. .................................................................- 65 -

Figure 3.13. The scattering cross-sections measured by SANS of the 18wt% gelatin hydrogels for several H\(_2\)O/D\(_2\)O concentrations (\(T = 20 \pm 2^\circ\)C). .................................................................- 67 -

Figure 3.14. Parameters obtained from SANS contrast measurements: (A) Correlation length from fitting results by equation 3.3. (B) SANS scattering cross section in Regime I at \(Q = 0.006 \text{ Å}^{-1}\). (C) SANS Scattering cross section in Regime I at \(Q = 0.054 \text{ Å}^{-1}\). (D) Sum of SANS Scattering intensity in the Q-range from \(Q = 0.005\) to \(0.05 \text{ Å}^{-1}\). .........- 68 -

Figure 3.15. Thermogravimetry (TG) on gelatin dry powders with a N\(_2\) flow.........- 70 -
Figure 3.16. (A) SANS scattering curves for 6 wt% gelatin in D$_2$O/H$_2$O mixtures (T = 20 ±2°C); (B) scattering intensity at different Q for samples in a mixture of D$_2$O/H$_2$O; (C) sum of scattering intensity in the Q-range from Q = 0.005 to 0.05 Å$^{-1}$ as function of heavy water content in D$_2$O/H$_2$O mixture. .................................................................- 71 -

Figure 3.17. SANS-VSANS scattered intensity versus scattering vector Q (Regime I, low Q for large structure) for gelatin 18 wt% in D$_2$O at different temperatures. ........- 72 -

Figure 3.18. Log-linear plot of SAXS scattered intensity versus scattering vector Q (Regime II, Large Q for small structure) for gelatin 18 wt% in D$_2$O at different temperatures. The inset picture is the SAXS intensity at Q = 0.01444 Å$^{-1}$ as a function of temperature. .................................................................................................- 73 -

Figure 3.19. Parameters from SAXS scattered intensity as a function of temperature for gelatin 18 wt % in D$_2$O at different Q. .................................................................................................- 74 -

Figure 3.2. The viscosity versus temperature results of gelatin hydrogels (18 wt% in H$_2$O). In the inset, T$_{g}$ is the so-gel transition temperature. The viscosity is measured by an oscillating rheometer with a fixed shear at 250 s$^{-1}$.................................................................- 50 -

Figure 3.20. Parameters from SAXS scattered intensity as a function of temperature for gelatin 18 wt% in D$_2$O. (A) The correlation length ($\xi$) and (B) the clustering strength (A/Q$^n$, Q = 0.12 Å$^{-1}$) obtained by fitting with equation 3.4. (C) The radius gyration (R$_g$) and (D) the high Q power law exponent (β) obtained by fitting with equation 3.1....- 75 -

Figure 3.21. SANS-VSANS scattering intensity versus scattering vector Q for gelatin 18 wt % in D$_2$O and D$_2$O with pD $\approx$ 1. The solid lines represent the fitting by Beaucage equation of equation 3.1(Q < 0.01 Å$^{-1}$) and correlation length model of equation 3.4 (Q > 0.01 Å$^{-1}$)..............................................................................................................- 77 -

Figure 3.22. SANS-VSANS scattered intensity versus scattering vector Q for gelatin 18 wt% in D$_2$O and D$_2$O with pD $\approx$ 13. The solid lines represent the fitting by Beaucage equation of equation 3.1(Q < 0.01 Å$^{-1}$) and correlation length model of equation 3.4 (Q > 0.01 Å$^{-1}$)..............................................................................................................- 78 -

Figure 3.23. SANS-VSANS scattered intensity versus scattering vector Q for gelatin 18 wt% in D$_2$O and salt solutions with different concentration and counterion valence. The inset represents the scattering intensity of samples at Q = 0.05 Å$^{-1}$ ...........................................- 80 -
Figure 3.24. Summary and schematic representation of the results of gelatin structure obtained from SANS/VSANS. \( \phi \) is the gelatin volume fraction. The term \( A/Q^b \) being the clustering strength and \( \xi \) being the correlation length model obtained from the correlation length model fitting results (equation 3.4). The \( R_g \) being the radius gyration obtained from the Beaucage equation fitting results (equation 3.1).

Figure 3.3. The loss modulus \( G'' \) and the storage modulus \( G' \) versus temperature of gelatin hydrogels (18 wt% in \( H_2O \)). \( T_g \) is the sol-gel transition temperature. The \( G'' \) and \( G' \) are measured by an oscillating rheometer with a fixed shear rate at 47 s\(^{-1}\).

Figure 3.4. The heat flux versus temperature DSC curves of (A) gelatin hydrogels (in \( H_2O \) as a function of hydrogel concentration (by weight) and (B) gelatin 18 wt% in \( D_2O \) and \( H_2O \). \( T_m \) is thermal transition midpoint.

Figure 3.5. The phase diagram of gelatin (in \( H_2O \)) as a function of concentration and temperature. \( T_m \) is the DSC thermal transition midpoint. \( T_{gel} \) is defined by the temperature at that the sol becomes significantly viscous and loses the flowability. \( T_{sol} \) is the temperature at that the gel becomes soft with flowability. The data points of the phase diagram were obtained by DSC, rheology, and microscopy measurements.

Figure 3.6. SANS/VSANS scattering profile of 18w% gelatin in \( D_2O \) is measured at \( T = 20 \pm 2^\circ \text{C} \). The light-blue solid line (\( Q < 0.01 \text{ Å}^{-1} \)) represents fitting by equation 3.1 of the Beaucage expression. The dark-blue solid line (\( Q > 0.01 \text{ Å}^{-1} \)) represents fitting by equation 3.3 of the fractal cylinder model. The dash-dot-red line represents a cylinder form factor with polydispersity of 0.2. The left inset picture is an AFM phase image showing gelatin large clusters (dried gelatin sample). The right bottom inset picture is a cylinder model for the gelatin triple helix.

Figure 3.7. Schematic representation of gelatin structure from larger scale to molecular level: (A) Gelatin large scale clusters constructed from the scattering data in regime I by a rapid \textit{ab initio} shape determination program DAMMIF\textsuperscript{135}. (B) Sketch of gelatin hydrogel structure with large clusters and gel network. (C) Cage like Gel matrix. (D) Gelatin triple-helix and random coil. (E) & (F) Macromolecular structure, a collagen-like triple-helical structure reconstructed from Chen et al\textsuperscript{119}. (Protein Database code 1CLG)

Figure 3.8. XRD patterns of gelatin dry films.
Figure 3.9. SANS scattered intensity versus scattering vector $Q$ for gelatin in $D_2O$ as a function of gelatin concentration ($T = 20 \pm 2^\circ C$). The scattering cross section was normalized with gel volume fraction $\phi$. At low $Q (< 0.002 \text{ Å}^{-1})$, VSANS data are also presented after rescaling. The solid lines represent fit by equation 3.1 and 3.4.

Figure 4.1. Schematic representation of experimental steps. (1) Synthesis of gelatin hydrogels. (2) The gelatin hydrogel films. (3) Magnetite mineralization in the hydrogel matrices.

Figure 4.10. SANS-VSANS scattering pattern of the gelatin-magnetite composites in pure $D_2O$ with the mineralization cycle times, zero (pure Gelatin 12 wt%), one, and two.

Figure 4.11. SANS scattered intensity versus scattering vector $Q$ for magnetite formation in gelatin in a mixture $D_2O/H_2O$ solvent of 28 vol.-% $D_2O$ and 72 vol.-% $H_2O$ ($T = 20 \pm 2^\circ C$). The composites were synthesised in the 18 wt% gelatin hydrogels. The solid line represents fit of the correlation length model of equation 4.3 (Fe10, Fe20, Fe50), Beaucage equation (High $Q$ feature of Fe100, Fe200) and Power law (Low $Q$ feature of Fe100 and Fe200, the scattering intensity is following a $Q^{-2.5}$ scattering).

Figure 4.12. Kratky plots of the SANS pattern from Figure 4.10 as a function of iron loading concentration.

Figure 4.13. Schematic representation of the shape dimension as a function of loading iron concentration.

Figure 4.14. XRD patterns of magnetite @ gelatin hybrid dry-gels as a function of initial iron soaked concentration. The round yellow mark reveals the peaks from the gelatin structure while the dash lines reveal the magnetite peaks.

Figure 4.15. Time-resolved SANS study of the magnetite mineralization kinetics in gelatin hydrogels in a mixture $D_2O/H_2O$ solvent of 28 vol.-% $D_2O$ and 72 vol.-% $H_2O$ ($T = 20 \pm 2^\circ C$). The mineralization were started in 18 wt% gelatin hydrogels. The solid line represents a fitting result by eq. 4.2 (30 s, 120 s, 270 s) and Beaucage equation (High $Q$ feature of 270 s, 420 s).

Figure 4.16. (A) Schematic representation of the magnetite mineralization kinetics in three steps. I) iron ion diffusion into the gel network, II) intermediate product formation
and attachment on the gelatin molecules, III) magnetite formation from the intermediate products. (B) A representative structure for FeIII(OH)₃ coordination by collagen. Note that three carbonyl/hydroxyl groups are providing an O·Fe salt bridge via one short (0.23 nm) and two weaker (0.26 nm) contacts. (C) FeII(OH)₂ cluster coordination by collagen leading to distorted/incomplete octahedral coordination of FeII (the number of coordinating water molecules from the solvent varies from 0 to 2). Atom colors: Fe (yellow), O (red / green for solvent), H (white), N(blue) and C(grey). The pictures (B) and (C) are the simulation results from our collaboration partner in Erlangen (AG Prof D. Zahn) and adapted from our publication¹⁷⁰.

**Figure 4.17.** Time-resolved SAXS study of the drying induced gelatin crystallization. The measurements were performed in vacuum with the sample gelatin 18 wt% in D₂O. (A) 3D plots of the TR-SAXS measurements. (B) Time-resolved SAXS scattering cross section against Q in a 2D plots.

**Figure 4.18.** Time-resolved SAXS parameters of the drying induced gelatin crystallization.

**Figure 4.19.** SAXS intensity dΣ/dΩ(Q) versus scattering vector Q for (made from gelatin 18 w%) wet and dry gel composites. The solid lines represent the fitting by equation 4.2.

**Figure 4.2.** (a) SANS-VSANS scattered intensity versus scattering vector Q for iron-loaded gelatin in D₂O (T = 20 ±2°C). In a Q range above 0.01 Å⁻¹, the solid line represents the best fit by correlation length model of equation 4.3. In the Q range below 0.01 Å⁻¹, the scattering curves were fitted by Beaucage expression of equation 4.2(Fe³⁺ ions consisting gels) and power law (Fe³⁺ ions free samples). Parameters from SANS-VSANS pattern of iron loaded gelatin samples: (b) the scattering intensity at Q=0.04 Å⁻¹, (c) the high-Q feature correlation length ξ, (d) the iron loaded hydrogel average cage size Rₘ, (e) the high Q power law exponent β (0.04 < Q < 0.4 Å⁻¹). The parameters from (b), (d) and (e) obtained from the SANS-VSANS results in Fig. 4.2 (a) while ξ are obtained from the fitting results from equation 4.3.

**Figure 4.20.** SAXS intensity dΣ/dΩ(Q) versus scattering vector Q for (made from gelatin 12 w%) wet and dry gel composites. The solid lines represent the fitting by equation 4.2.
Figure 4.21. SAXS intensity $d\Sigma/d\Omega(Q)$ versus scattering vector $Q$ for magnetite powders. ................................................................. - 118 -

Figure 4.22. (A) SAXS intensity $d\Sigma/d\Omega(Q)$ versus scattering vector $Q$ for chiton tooth at different positions. ................................................................................. - 119 -

Figure 4.23. Summary and schematic representation of the results of superparamagnetite mineralization in gelatin hydrogels in D$_2$O and D$_2$O/H$_2$O 28 V/V% obtained by SANS/VSANS. .......................................................................................... - 121 -

Figure 4.3. Schematic representation of gelatin hydrogel structure changes with Fe$^{3+}$ and Fe$^{2+}$ loading. ................................................................................................................... - 96 -

Figure 4.5. AFM images of the dry composite films. (A) and (C) are the phase contrast images. (B) and (D) are the amplitude images. ......................................................................................... - 98 -

Figure 4.6. Magnetic properties of the synthesized hybrid materials.\textsuperscript{170} a) Magnetization curves of a dried ferrogel at 2 K and 293 K. b) ZFC-FC curves as a function of temperature. .................................................................................................................. - 100 -

Figure 4.7. XRD of the dry gelatin and gelatin-magnetite composites. .................................. - 101 -

Figure 4.8. SANS-VSANS scattering pattern of the gelatin-magnetite composites (sample prepared from 18 wt% gelatin hydrogels) in pure D$_2$O and a mixed D$_2$O/H$_2$O solvent of 28 vol% D$_2$O and 72 vol% H$_2$O. The blue solid lines represent the fitting of the Beaucage expression (equation 4.2) at high Q and power law in the low Q. The red solid lines represent the fitting of the correlation length model (equation 4.3) at high Q and power law in the low Q. In a box of the inset picture there is a shape reconstruction result from the scattering of the sample by using DAMMIF.\textsuperscript{135} ....................................................................... - 102 -

Figure 4.9. SANS-VSANS scattering pattern of the gelatin-magnetite composites in pure D$_2$O, iron-loaded gel and pure gelatin 18 wt% in D$_2$O. The solid lines represent the fitting by correlation length model of equation 4.3($Q > 0.01$ Å$^{-1}$), Beaucage expression (equation 4.2) of pure gelatin 18 wt% in D$_2$O ($Q < 0.01$ Å$^{-1}$) and power law of gelatin-magnetite composites and iron-load gel in D$_2$O ($Q < 0.01$ Å$^{-1}$). ........................................................................ - 104 -

Figure 4.1. Magnetic regimes of magnetite as a function of their size (superparamagnetic, stable single domain, multi-domain). ................................................................. - 123 -
Figure 5.10. Time-resolved SANS study of the magnetite mineralization kinetics in the presence of gelatin hydrogels (18 wt%) in a mixture D$_2$O/H$_2$O solvent of 28 vol.% D$_2$O and 72 vol.% H$_2$O (T = 20 ±2°C). The inset is a gelatin large particle shape construction result from the scattering data by a rapid *ab initio* shape determination program DAMMIF$^{135}$. The solid line represents the fitting results by the Beaucage expression (equation 5.1). .............................................................. - 141 -

Figure 5.11. Parameters obtained from the TR SANS-VSANS results. On the right is the possible magnetite formation mechanisms. .......................................................... - 141 -

Figure 5.12. XRD patterns of magnetite-gelatin hybrid dry gels as a function of mineralization time. ........................................................................................................ - 142 -

Figure 5.13. VSANS study of the magnetite formation in the presence of gelatin hydrogels in 28 wt% D$_2$O (T = 20 ±2°C) with and without magnetic field. .......... - 143 -

Figure 5.14. Summary and schematic representation of the results of large magnetite nanoparticles formation in the presence of gelatin hydrogels in D$_2$O and D$_2$O/H$_2$O 28 V/V% obtained by SANS/VSANS............................................................. - 145 -

Figure 5.2. Schematic representation of experimental steps. (1) Synthesis of Gelatin Hydrogels. (2) The gelatin hydrogel films. (3) Magnetite mineralization in the hydrogel matrices.................................................................................................. - 127 -

Figure 5.3. (a) SANS-VSANS scattered intensity versus scattering vector Q for ironII-loaded gelatin in D$_2$O (T = 20 ±2°C). The solid line represents the best fit by equations 5.1 (Beaucage equation, for Q > 0.01 Å$^{-1}$) and 5.2 (correlation length model, for Q > 0.01 Å$^{-1}$). Parameters from SANS-VSANS pattern of iron(II)-loading and iron-free gelatin samples are listed in the inset table...................................................... - 130 -

Figure 5.4. A) Temperature dependence of magnetization (M-H) measured at 2 K and 293 K. B) FC and ZFC curves plotted as a function of temperature. The magnetic result data is a representation from Maria S.$^{187}$ ................................................................. - 132 -

Figure 5.5. TEM results of the formed large magnetite nanoparticles. (A) Composites prepared from an iron source of 0.2 M Fe$^{2+}$ and (B) repeated mineralization process for 4 reaction cycles. (C) HR-TEM image of a composite sample after one reaction cycle. (iron II source of 0.2 M Fe$^{2+}$) and the FFT image (D). The data is a representation and adapted from Maria S.$^{187}$ .................................................................................................... - 133 -

- 203 -
Figure 5.6. A typical sample synthesized by the partial oxidation method, by which 10 wt% gelatin hydrogels with FeSO$_4$ (0.3 M) are treated in a KOH (0.1 M), KNO$_3$ (0.25 M) solutions in 4 reaction cycles. (a) SANS-VSANS scattering intensity against Q measured in 28 vol% D$_2$O, the solid line is the best fitting by equation 5.1. (b) TEM image of the large magnetite nanoparticles-gelatin composites.

Figure 5.7. SANS-VSANS scattering pattern of the gelatin-magnetite composites in pure D$_2$O. The sample was prepared from 10 wt% gelatin hydrogels with FeSO$_4$ (0.3 M) and treated with a KOH (0.1 M), KNO$_3$ (0.25 M) solutions in 4 reaction cycles. The gelatin hydrogels 12 wt% in D$_2$O was plotted for comparison. The solid lines are the best fitting by equation 5.1 and 5.2 as well as the power law for the composites at very low Q.

Figure 5.8. Sample synthesized by the partial oxidation method, by which 10 wt% gelatin hydrogel with FeSO$_4$ (0.2 M) are treated in KOH (0.1 M), KNO$_3$ (0.25 M and 0.5 M) solutions in 4 reaction cycles.

Figure 5.9. Sample synthesized by the partial oxidation method, by which 10 wt% gelatin hydrogel with FeSO$_4$ (0.2 M and 0.3 M) are treated in KOH (0.1 M), KNO$_3$ (0.5 M) solutions in 4 reaction cycles.

Figure 6.1. SANS-VSANS scattering intensity against Q of a typical sample synthesized by a two-step method, by which 18 wt% gelatin hydrogels with FeCl$_2$ (0.2 M) were treated in KOH (0.1 M), KNO$_3$ (0.5 M) solution. The sample was placed in a 28 vol% D$_2$O solution. The solid line is the best fitting by the Beaucage equation.

Figure 6.2. SANS-VSANS scattering intensity against Q of the sample measured at different time stages, by which 18 wt% gelatin hydrogels with FeCl$_2$ (0.2 M) were treated in KOH (0.1 M), KNO$_3$ (0.5 M) solution. The sample was placed in a 28 vol% D$_2$O solution. The inset $R_g$ plots obtained from the fitting of the Beaucage equation.

Figure 6.3. XRD patterns of magnetite-gelatin hybrid dry gels as a function of mineralization time.

Figure 6.4. SANS-VSANS scattering intensity against Q of the sample measured at different time stages, by which 18 wt% gelatin hydrogels with FeCl$_2$ (0.2 M) were treated in KOH (0.1 M), KNO$_3$ (0.25 M) solution. The sample was placed in a 28 vol%
D$_2$O solution. The inset $R_g$ plot is obtained from the fitting of the Beaucage equation. ...

**Figure 6.5** Summary and schematic representation of the results of two-step magnetite nanoparticles formation in the presence of gelatin hydrogels in D$_2$O/H$_2$O 28 V/V% obtained by SANS/VSANS. ..........................................................- 153 -

**Figure 7.1.** Schematic representation of experimental steps. (1) Nacre demineralization. (2) Infiltration with gelatin. (3) Magnetite Mineralization in the hybrid organic matrices. ..........................................................- 157 -

**Figure 7.2.** (a) The nacre tablet structure and (b) SANS scattering geometry on the nacre and nacre organic matrix..........................................................- 159 -

**Figure 7.3.** SANS macroscopic cross-section $d\Sigma/d\Omega$ versus scattering vector $Q$ for a 1 mm thick piece of nacre in air and a demineralized nacre matrix in D$_2$O ($T = 20 \, ^\circ C$). The neutron beam is parallel to the nacre/nacre organic matrix c-axis (perpendicular to the sample surface). At low $Q$ (<0.002 Å$^{-1}$) VSANS data are also presented after rescaling. The solid line represents a fit of the Beaucage equation$^{104}$ and correlation length model$^{105}$ ($Q > 0.003 \, \text{Å}^{-1}$). The insets are 2D scattering of the VSANS large structure. ..........................................................- 162 -

**Figure 7.4.** SAXS scattered intensity versus scattering vector $Q$ for dry Nacre organic matrix. The solid line represents a fractal cylinder model$^{137,138}$ fitting. On the top-right is a WAXS result and a schematic representation of the dry matrix structure (left).- 164 -

**Figure 7.5.** The hierarchical structure of nacre and nacre organic matrix. (a) Shells of *Haleotis laevigata*. (b) SEM image of the nacre platelet structure. (c) A cartoon of nacre platelet structures (on top view). (d) A fresh nacre organic matrix piece. (e) TEM result on the chitin sheet structure. (f) A cartoon of nacre platelet structures and chitin layer (on the side view). (g) $\alpha$-chitin texture. (h) Chemical structure of chitin............................- 165 -

**Figure 7.6.** SANS macroscopic cross-section $d\Sigma/d\Omega$ versus scattering vector $Q$ for (1) nacre powders, (2) 1 mm thick piece of nacre in air and a demineralized nacre matrix in D$_2$O ($T = 20 \, ^\circ C$) by (3) strong acetic acid and (4) EDTA. The neutron beam is parallel to the nacre/nacre organic matrix c-axis (perpendicular to the sample surface). At low $Q$ (<0.002 Å$^{-1}$) VSANS data are also presented after rescaling..............................................- 166 -
**Figure 7.7.** VSANS scattering patterns of nacre organic matrix and gelatin-nacre organic matrix hybrids in D$_2$O..........................................................- 166 -

**Figure 7.8.** SANS and VSANS scattering patterns of magnetite in a gelatin-chitin composite and of a ferrogel in a mixed D$_2$O/H$_2$O solvent of 28 vol% D$_2$O and 72 vol% H$_2$O. The solid lines represent the fitting of the Beaucage expression (equation 7.1). ......- 167 -

**Figure 8.1** Summary and schematic representation of the chapter organisation. ....- 172 -

**Figure 8.2.** Scheme presentation of structural characterization of the hybrid materials using small angle scattering methods. ..........................................................- 176 -

**Figure B1.1.** Synthesis of Magnetite in D$_2$O and H$_2$O by coprecipitation of Fe$^{2+}$ and Fe$^{3+}$ ions in a non-oxidation condition. ..........................................................- 179 -

**Figure B1.2.** XRD patterns of magnetite coprecipitation at pH 11 in D$_2$O and H$_2$O conditions...........................................................................................................- 180 -

**Figure B1.3.** TEM results of magnetite coprecipitation at pH 11 in D$_2$O (A) and H$_2$O (B) conditions ...........................................................................................................- 180 -

**Figure B2.1.** TEM results of magnetite coprecipitation at pH 11 in gelatin hydrogels in D$_2$O (A) and H$_2$O (B). ...........................................................................................................- 180 -

**Figure C 1.1.** A picture of the SANS instrument KWS-1.$^{92}$.............................................- 181 -

**Figure C 1.2.** Sample stage, Hexapod of KWS-1.$^{90}$ ...........................................................- 182 -

**Figure C 1.3.** Sample position of KWS-1.$^{90,202}$ .............................................................- 182 -

**Figure C 2.1.** Q-range of the KWS-3 diffractometer for all available configurations (#0 to #5) is plotted. For comparison, the Q-range of a classical pine-hole SANS instrument is plotted too.$^{203}$ ...........................................................................................................- 184 -

**Figure C 3.1.** Hellma 404-QX cells, with path length of 0.5, 1, 2 and 5mm.............- 186 -

**Figure C 3.2.** Sandwich cells, with path length of 0.5, 1, 2 and 5mm.............- 186 -

**Figure D 1.1.** Sample cells for SAXS experiments. .........................................................- 187 -

**Table 2.1.** Comparison of the (coherent) scattering length for neutrons and x-rays for a selection of elements. The area of the colored circles represents the scattering length.
All of this data was taken from the Special Features section of neutron scattering lengths and cross sections of the elements and their isotopes in Neutron News.  

**Table 2.2.** Technical data in detail for the two SAXS instruments.  

**Table 2.3.** An assortment of Porod law behaviors for different shape objects. The pictures are edit and represent from B. Hammouda and his lecture note. The red circle is the scattering probed regime.  

**Table 3.1.** Parameters of Gelatin hydrogels in D$_2$O obtained from the SANS/VSANS data.  

**Table 3.2.** Parameters of Gelatin hydrogels in D$_2$O obtained from the SANS/VSANS data.  

**Table 3.3.** Parameters of Gelatin solutions (with acetate buffer, pD=5.2) in D$_2$O obtained from the SANS data.  

**Table 3.4.** Parameters of Gelatin hydrogels in D$_2$O/H$_2$O mixture obtained from SANS contrast measurements.  

**Table 3.5.** Parameters of Gelatin hydrogels in D$_2$O with different pD values obtained from the SANS- VSANS data.  

**Table 3.6.** Parameters of Gelatin hydrogels in D$_2$O with different concentration and counterion valence obtained from the SANS- VSANS data.  

**Table 4.1.** Parameters of iron ions-loaded Gel in D$_2$O obtained from the SANS- VSANS data and fitting results.  

**Table 4.2.** Parameters of Magnetite-Gel in D$_2$O/H$_2$O (D$_2$O Vol 28%).  

**Table 4.5.** Parameters of the gel-composites in D$_2$O/H$_2$O (D$_2$O Vol 28%) obtained from the kinetic measurement. The R$_g$ and correlation length obtained from fitting results of equation 4.2 and 4.3.  

**Table A1.** Radii of gyration of some homogeneous objects.  

**Table A2.** List of common matter density and their scattering length densities in the thesis.

209


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