Synthesis and Characterization of Polymer-Mediated Biomimetic Calcium Carbonate Materials

A DISSERTATION
Submitted in Partial Fulfillment of the Requirement for the Doctor Degree Dr. rer. nat.
to the Faculty of Natural Sciences

University of Ulm, Germany

By
Ranjith Krishna Pai
From India
2005
Synthesis and Characterization of Polymer-Mediated Biomimetic Calcium Carbonate Materials

DISSERTATION
zur Erlangung des
Doktorgrades Dr. rer. nat.
der Fakultät für Naturwissenschaften
der Universität Ulm

vorgelegt von
Ranjith Krishna Pai
aus Indien
2005
Amtierender Dekan: Prof. Dr. Klaus-Dieter Spindler

1. Gutachter: Prof. Dr. Othmar Marti

2. Gutachter: Pof. Dr. Katherina Landfester

Tag der Promotion: 14.10.2005
Dedicated to my ever loving Parents...
Contents

Abstract...........................................................................................................VIII
Zusammenfassung.......................................................................................... XI
List of Nomenclature..................................................................................... XV

1. History of Nucleation and Growth of Biominerals......................1
   1.1. Biomineralization - an overview.................................................. 1
   1.2. Controlled nucleation of biological minerals............................. 8
       1.2.1. Oriented nucleation induced by Organic Surfaces........... 11
       1.2.2. Shaping of Biogenic minerals........................................ 13
   1.3. Biomimicry.................................................................................... 15
   1.4. Theory.......................................................................................... 17
       1.4.1. Equilibrium and Supersaturation..................................... 17
       1.4.2. Ostwald-Freundlich relation............................................. 22
       1.4.3. Nucleation................................................................. 26
       1.4.4. Crystal Growth............................................................ 34
       1.4.5. Secondary Changes...................................................... 37
       1.4.6. Influence of Additives..................................................... 39
   1.5. Review of Calcium Carbonate..................................................... 43
       1.5.1. Polymorphism............................................................... 44
       1.5.2. Anhydrous polymorphs................................................. 44
       1.5.3. Amorphous and Hydrated polymorphs........................... 45
       1.5.4. Phase Transformation.................................................... 47
       1.5.5. Crystal Growth and Dissolution..................................... 48
       1.5.6. Biomimetic Methods for Synthesis of CaCO₃.................. 50
       1.5.7. Easy Approach for the Synthesis of CaCO₃.................. 52

2. CaCO₃ Crystal Modifications Using Water-soluble Polymers Based on AMPS Monomer .................................................. 53
   2.1. Introduction.................................................................................. 53
       2.1.1. Direction of research..................................................... 53
       2.1.2. Performance properties of water soluble polymers........ 55
       2.1.3. Synthetic strategy......................................................... 56
2.2. Experimental Section ................................................................. 58
  2.2.1. Materials ........................................................................... 58
  2.2.2. Methods ........................................................................... 58
2.3. Results and Discussion ............................................................ 62
  2.3.1. Crystals precipitated in the polymer-free control experiments ........................................................................... 62
  2.3.2. Crystals precipitated in the presence of terpolymer with varying mole percentage of monomeric units .................. 63
  2.3.3. Crystals precipitated in the presence of terpolymer with different carbonate salts ......................................................... 67
  2.3.4. Crystals precipitated in the presence of terpolymer with the replacement from SA to NVP unit .................................. 68
  2.3.5. Crystals precipitated in the presence of copolymer with the replacement from AMPS to NaAMPS unit .................... 69
  2.3.6. Crystals precipitated in the presence of copolymer with varying mole percentage of monomeric units .................. 72
  2.3.7. Water-soluble terpolymer direct the hollow triangular cones of packed calcite needles .................................................. 73
  2.3.8. Crystals precipitated in the presence of terpolymer with poly-L-aspartic acid ............................................................... 78
  2.3.9. Formation of different morphologies of calcium carbonate: Role of Poly (AM-NaAMPS-SA) under the control of various additives ........................................................... 86
2.4. Conclusion .................................................................................. 108
3. Miniemulsion-based Synthesis of Calcium Carbonate ........... 110
  3.1. Introduction ............................................................................ 110
    3.1.1. Methods of Synthesis of Nanoparticles ............................... 112
    3.1.2. Motivation of the study ...................................................... 115
    3.1.3. Miniemulsion .................................................................. 117
    3.1.4. Ostwald ripening and Collision process .............................. 119
    3.1.5. Transport Mechanism ....................................................... 122
    3.1.6. Surfactant Selection .......................................................... 124
  3.2. Experimental Section ............................................................... 124
    3.2.1. Materials ........................................................................ 124
3.2.2. Preparation of Calcium carbonate ........................................ 124
3.3. Results and Discussion .......................................................... 126
   3.3.1. Synthesis of Amorphous Calcium Carbonate ................. 126
      3.3.1.1. Transformation from CaCO₃:H₂O via CaCO₃:6H₂O to
               vaterite modifications: Characterization by FT/IR and
               Raman spectroscopy .............................................. 138
   3.3.2. Synthesis of CaCO₃ using poly [(ethylene-co-butylene)-b-
       (ethylene oxide)] and also in combination with magnesium
       chloride and N-acetyl-L-glutamic acid ............................ 142
      3.3.2.1. CaCO₃ precipitation using poly [(ethylene-co-butylene)-
               b-(ethylene oxide)] ............................................ 143
      3.3.2.2. CaCO₃ precipitation using [(ethylene-co-butylene)-b-(ethylene
               oxide)] in combination with magnesium chloride ........ 146
      3.3.2.3. CaCO₃ precipitation using [(ethylene-co-butylene)-b-(ethylene
               oxide)] in combination with N-acetyl-L-glutamic acid ...... 153
3.4. Conclusion ............................................................................... 156

4.   AFM Investigation on CaCO₃ deposits of Porcellio scaber ... 161
   4.1. Introduction ........................................................................... 161
      4.1.1. Direction of research .................................................. 161
      4.1.2. Material and Methods ............................................... 166
      4.1.3. The dynamic SFM mode ............................................. 167
      4.1.4. Roughness Analysis .................................................. 168
   4.2. Results .................................................................................. 170
      4.2.1. Effect of Methanol .................................................... 170
      4.2.2. Concentric Growth Mechanism ................................. 171
   4.3. Discussion .............................................................................. 180
   4.4. Conclusion ............................................................................. 182

5.   Summary and Outlook .............................................................. 183
   List of Figures and Tables .......................................................... 193
   Bibliography .............................................................................. 198
   Declaration ................................................................................. 212
   Acknowledgement, Publications, Posters and Conferences ....... 213
Abstract

Synthesis of inorganic nanoparticles is currently recognized as an important theme in nanoscience. Calcium carbonate (CaCO₃) nanoparticles (<100 nm) have many unique properties compared to regular CaCO₃ particles. CaCO₃ is an important mineral, exhibits different shapes and colors, depending on the conditions at its formation and the nature of additives present during growth. In addition to its importance as a major geological deposit, CaCO₃ is used in a variety of applications; from a filler in paints and toothpaste to component in cements and optical instruments. Calcite deposits can also cause problems; furring in kettles, blockages in industrial piping and even in living organisms. By controlling the growth of calcite, it may be possible to refine its production and control its problematic deposition.

Many living organisms are well able to synthesize highly optimized composite materials by means of template controlled mineralization as in pearls, shells, etc. For example, amorphous calcium carbonate (ACC) deposits of Porcellio scaber (land-based woodlice) are built of numerous spherules associated with an organic matrix that consists of radial and concentric elements, suggesting that the organic components function as a template for spherule formation. Recent efforts to duplicate the natural process of biomineralisation in the laboratory have come to be known as biomimetic methods for synthesis. An understanding of the mechanism involved in such a matrix-mediated synthesis has been recognized to be of great potential in the production of engineered materials. In the present study,
**Abstract**

*Porcellio scaber* is used as a model to investigate the dissolution behavior of biogenic ACC by atomic force microscopy which provides a new way to investigate the stabilizing properties of matrix components.

Biological systems manage to control crystal growth to high degrees of specificity by using a wide range of natural additives; from basic metal ions to complex organic polymers. Organic polymers control crystal growth, habit and morphology through molecular interactions at the nanoscale. Biopolymers can also adsorb onto crystal surfaces, altering the stability of that face and hence affecting the growth rate. These organic regulators are often identified as polymers that contain repeat units of distinct peptides, or amino acids, in their primary sequence. The question which remains to be answered is the nature of the crystal-organic interactions that mediate these effects. In this context, an examination of how CaCO₃ growth is affected by water-soluble polymers and low molecular weight additives will provide further insight and information.

Proteins and polypeptides possess carboxylic acid groups in their side chains and are known to shape biogenic CaCO₃. Because of this, related additives were selected which contain acidic groups available to interact with other molecules or surfaces. It is widely accepted that the acidic functional groups adsorb onto the surface of calcite. It is generally proposed that mimetic substitution by the carboxylic acid groups for the carbonate ions on the calcite surface slows the growth of the higher energy, faster growing faces, hence altering the crystal habit and morphology. Organisms generally utilize complex mixtures of crystal modifiers rather than simple additives to generate the complex biomaterials observed. To begin to address this, I
have investigated the effect of water-soluble polymers with amide, carboxylic and sulphonic acid functionalities, on the crystallization behavior of CaCO₃. The polymers used were acrylamide based on acrylamido methyl propane sulphonic acid with varying monomer compositions and monomeric units. For the desired purpose of ion binding, several low molecular weight additives such as simple amino acids, peptides, proteins, inorganic salts were also examined in combination with polymers.

Here the synthesis of CaCO₃ was followed by two synthetic routes: double decomposition reaction (a simple route, but it is difficult to control the shape and modification of CaCO₃) and miniemulsion method (CaCO₃ synthesis was performed in $10^{18} - 10^{20}$ nanocompartments which are separated from each other by a continuous phase). ACC is a fascinating form of CaCO₃ that may well be of much interest to materials science and biomineralization. ACC in its pure form is highly unstable, yet some organisms produce stable ACC, and cases are known in which ACC function as a transient precursor of more stable crystalline CaCO₃. By using miniemulsion technique, I have synthesized ACC nanoparticles which are stable in solution and also in the dry state for a several days.
Zusammenfassung


Viele lebende Organismen sind fähig, hoch optimierte Kompositmaterialien wie Perlen, Muschelschalen etc. mittels templatkontrollierter Mineralisation zu synthetisieren. Zum Beispiel sind die röntgenamorphen Calciumcarbonat (ACC)-Ablagerungen von *Porcellio scaber* (Kellerassel) aus zahlreichen Sphärolithen aufgebaut, die eine organische Matrix aus radialen und konzentrischen Elementen enthält, was vermuten läßt, daß die organischen Komponenten als Templat für die Sphärolithe fungieren. Neuere Versuche, natürliche Synthesemethode im Labor nachzuahmen, wurden unter dem Begriff
biomimetische Synthesemethoden bekannt. Das Verständnis des an
der „Matrix-vermittelten-Synthese“ beteiligten Mechanismus wird als
Voraussetzung für die Herstellung vielfältiger Materialien angesehen.
In dieser Arbeit wird *Porcellio scaber* als Modell zur
rasterkraftmikroskopischen Untersuchung des Lösungsverhaltens
sternaler ACC-Reservoire verwendet, um so ein besseres Verständnis
der stabilisierenden Wirkung der Matrixkomponenten zu erreichen.

Solche biologischen Systeme können das Kristallwachstum
durch die Verwendung einer Vielzahl natürlicher Additive, von
einfachen Metallionen bis zu komplexen organischen Polymeren, mit
großer Genauigkeit kontrollieren. Organische Polymere beeinflussen
das Wachstum, den Habitus und die Morphologie der Kristalle durch
zwischenmolekulare Wechselwirkung auf der Nanoskala. Biopolymere
können auch auf Kristalloberflächen adsorbiert werden, damit die
Stabilität dieser Oberfläche verändern und die Wachstumsrate
beeinflussen. Diese organischen Regulatoren werden oft als Polymere
identifiziert, die sich wiederholende Motive bestimmter Peptide oder
Aminosäuren in ihrer primären Sequenz enthalten. Die Frage nach der
Art dieser „kristall-organischen“ Wechselwirkung, die diese Effekte
bewirkt, ist unbeantwortet. In diesem Kontext wird eine Überprüfung,
wie das Calcit-Wachstum durch wasserlösliche Polymere und
niedermolekulare Additive beeinflusst wird, weitere Aufklärung und
Information liefern.


Die Synthese des CaCO₃ wurde auf zwei verschiedenen Wegen versucht: über eine doppelte Zersetzungsreaktion (ein einfacher Weg, aber es ist schwierig, die Gestalt und die Modifikation des CaCO₃ zu
kontrollieren) und über die Miniemulsionstechnik (CaCO₃ Synthese wurde durchgeführt in 10¹⁸ - 10²⁰ Nanokompartimenten, die durch eine kontinuierliche Phase voneinander getrennt waren). Das ACC ist eine faszinierende Form des CaCO₃, das von hohem Interesse für Materialwissenschaften und Biomineralisation sein kann. ACC ist in seiner reinen Form sehr labil, allerdings produzieren einige Organismen stabiles ACC und es sind einige Fälle bekannt, in denen ACC als eine vorübergehende Vorstufe des stabilen kristallisierten CaCO₃ dient. Mit der Miniemulsionstechnik konnten in dieser Arbeit ACC Nanoteilchen synthetisieren werden, die in Lösung stabil und im getrockneten Zustand zumindest einige Tage stabil waren.
# Nomenclature

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Description</th>
<th>Unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Constant(s)</td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>Area</td>
<td>[m²]</td>
</tr>
<tr>
<td>a</td>
<td>Area</td>
<td>[m²]</td>
</tr>
<tr>
<td>C</td>
<td>Constant(s)</td>
<td></td>
</tr>
<tr>
<td>c</td>
<td>Concentration</td>
<td>[kmol m⁻³]</td>
</tr>
<tr>
<td>a</td>
<td>Activity</td>
<td>[kmol m⁻³]</td>
</tr>
<tr>
<td>d</td>
<td>Impurity density</td>
<td>[m⁻²]</td>
</tr>
<tr>
<td>f</td>
<td>Correction factor</td>
<td>[-]</td>
</tr>
<tr>
<td>G</td>
<td>Gibbs free energy</td>
<td>[kg m² s⁻²]</td>
</tr>
<tr>
<td>g</td>
<td>Crystal growth rate</td>
<td>[m s⁻¹]</td>
</tr>
<tr>
<td>H</td>
<td>Enthalpy</td>
<td>[kg m² s⁻²]</td>
</tr>
<tr>
<td>I</td>
<td>Ionic strength</td>
<td>[kmol m⁻³]</td>
</tr>
<tr>
<td>J</td>
<td>Nucleation rate</td>
<td>[m⁻³ s⁻¹]</td>
</tr>
<tr>
<td>K</td>
<td>Equilibrium constant(s)</td>
<td></td>
</tr>
<tr>
<td>k</td>
<td>Boltzmann constant</td>
<td>[kg K⁻¹ s⁻²]</td>
</tr>
<tr>
<td>L</td>
<td>Length</td>
<td>[m]</td>
</tr>
<tr>
<td>M</td>
<td>Molar mass</td>
<td>[kg kmol⁻¹]</td>
</tr>
<tr>
<td>m</td>
<td>Mass</td>
<td>[kg]</td>
</tr>
<tr>
<td>N</td>
<td>Mole number</td>
<td>[kmol⁻¹]</td>
</tr>
<tr>
<td>N</td>
<td>Particle concentration</td>
<td>[m⁻³]</td>
</tr>
<tr>
<td>n</td>
<td>Moles</td>
<td>[kmol]</td>
</tr>
<tr>
<td>P</td>
<td>Pressure</td>
<td>[kg m⁻¹ s⁻²]</td>
</tr>
<tr>
<td>R</td>
<td>Gas constant</td>
<td>[kg m² kmol⁻¹ k⁻¹ s⁻²]</td>
</tr>
<tr>
<td>R</td>
<td>Rate</td>
<td>[kmol m² s⁻¹]</td>
</tr>
<tr>
<td>r</td>
<td>Radius</td>
<td>[m]</td>
</tr>
<tr>
<td>S</td>
<td>Entropy</td>
<td>[kg m² K⁻¹ s⁻²]</td>
</tr>
<tr>
<td>S</td>
<td>Supersaturation</td>
<td>[-]</td>
</tr>
<tr>
<td>s</td>
<td>Specific surface area</td>
<td>[m⁻¹]</td>
</tr>
<tr>
<td>Symbol</td>
<td>Description</td>
<td>Unit</td>
</tr>
<tr>
<td>--------</td>
<td>-----------------------------------</td>
<td>------------</td>
</tr>
<tr>
<td>T</td>
<td>Temperature</td>
<td>[K]</td>
</tr>
<tr>
<td>t</td>
<td>Time</td>
<td>[s]</td>
</tr>
<tr>
<td>U</td>
<td>Energy</td>
<td>[kg m² s⁻²]</td>
</tr>
<tr>
<td>V</td>
<td>Volume</td>
<td>[m³]</td>
</tr>
<tr>
<td>V</td>
<td>Layer velocity</td>
<td>[m s⁻¹]</td>
</tr>
<tr>
<td>z</td>
<td>Charge</td>
<td>[-]</td>
</tr>
<tr>
<td>α</td>
<td>Effectiveness factor</td>
<td>[-]</td>
</tr>
<tr>
<td>γ</td>
<td>Activity coefficient</td>
<td>[-]</td>
</tr>
<tr>
<td>μ</td>
<td>Chemical potential</td>
<td>[kg m² kmol⁻¹ s⁻²]</td>
</tr>
<tr>
<td>ν</td>
<td>Stoichiometric coefficient</td>
<td>[-]</td>
</tr>
<tr>
<td>θ</td>
<td>Wetting angle</td>
<td>[-]</td>
</tr>
<tr>
<td>ρ</td>
<td>Density</td>
<td>[kg m⁻³]</td>
</tr>
<tr>
<td>ρ</td>
<td>Step curvature radius</td>
<td>[m]</td>
</tr>
<tr>
<td>σ</td>
<td>Interfacial energy</td>
<td>[kg s⁻²]</td>
</tr>
<tr>
<td>σ'</td>
<td>Edge free energy</td>
<td>[kg m s⁻²]</td>
</tr>
<tr>
<td>ξ</td>
<td>Advancement variable</td>
<td>[kmol]</td>
</tr>
</tbody>
</table>
Chapter 1

History of Nucleation and Growth of Biominerals

1 Introduction

The process of Biomineralization and Biomimicry is well documented and studies on Calcium Carbonate precipitation from aqueous solutions have been performed by a large number of authors. Literature relevant to this work has been gathered and summarized in this chapter.

1.1 Biomineralization - an overview

The study of biomineralization offers valuable insights into the scope and nature of materials chemistry at the inorganic-organic interface (Calvert et al. 1998, Mann et al. 1992, Heuer et al. 1992, Addadi et al. 1992, Mann et al. 1993). Biological systems are replete with examples of organic supramolecular assemblies (double and triple helices, multisubunit proteins, membrane-bound reaction centers, vesicles, tubules, etc.), some of which (collagen, cellulose, and chitin) extend to microscopic dimensions in the form of hierarchical structures. Of interest here is the manner in which these organic architectures can be associated with inorganic solids to give unique and exquisite biominerals in which the structure, size, shape, orientation, texture, and assembly of the mineral constituents are precisely controlled. The intimate association of inorganic and organic phase is a hallmark of biomineralization. In many cases, the integration is at the
superstructural level, where mineral particles and biopolymers are organized to give composites of unusual strength and toughness. In other systems the association of inorganic and organic components is more subtle. Surprisingly, a spine of a sea urchin, with its elaborate (noncrystallographic) texture and porosity, generates a highly ordered, single crystal X-ray diffraction pattern, yet it contains 0.02 wt% of protein (Berman et al. 1993).

Much is known about the ultrastructures of biominerals and how they vary in different organism, but precise details about the molecular interactions governing their construction have yet to be revealed. The aqueous precipitation of many minerals is a relatively straightforward laboratory procedure, but while it may be relatively simple to grow crystal of compound, controlling the size, shape, orientation, and assembly of these crystals, as is typical of many biominerals, is a much more complex task. Because the fabrication of biological ultrastructures is ultimately governed by the nature of the molecular interaction occurring at solid-liquid and solid-solid interfaces, biominerals represent archetypes of how synthetic strategies can be orchestrated towards the construction of organized systems (Mann et al. 1996). At the nanoscale level, biomineralization involves the molecular construction of discrete, self-assembled, organic supramolecular systems (micelles, vesicles, etc.) that are used as preorganized environment for controlling the formation of finely divided inorganic materials (~ 1-100 nm in size). The fabrication of consolidated biominerals, such as bone and teeth, also involves the construction of preorganized organic frameworks, but the length scale is greater (micrometers) and the matrix is often polymeric (collagen, enamel tubules etc.). The building of discrete or extended architectures
in biomineralization often involves hierarchical processing in which the molecular-based construction of organic assemblies is used to provide frameworks for the synthesis of organized materials, which in turn are exploited as prefabricated units in the production of higher order complex microstructures. The formation of calcified minerals (as amorphous modification) in the land-based woodlice (*Porcellio scaber*) illustrates this process. Terrestrial isopods store calcium in a variety of storage sites (Ziegler, A. 2003). The most common among these are the sternal CaCO₃ deposits located within the ecdysial gap between the epithelium and the old cuticle of the first four anterior sternites (Ziegler and Miller, 1997; Ziegler, A. 2003) (Figure 1.1a).

**Figure 1.1. The sternal CaCO₃ deposits of *Porcellio scaber*.** The sternal CaCO₃ deposits of *Porcellio scaber* in the late premolt stage. (a) Light micrograph of the deposits outlining deposits of the sternites 2-4. The smaller first sternite is hidden by the head. (b) Knife polished sagittal face of a fully developed deposit treated with Ches buffer pH 10 for 5min. (dsl) distal spherular layer, (psl) proximal spherular layer, (hl) homogenous layer. (c, d) Cleaved surfaces of untreated deposits showing that the material within hl and the spherules within psl (c) and dsl (d) appear solid. Source: Ziegler and Miller, 1997; Ziegler, 2003.
Electron spectroscopic diffraction analysis and high resolution synchrotron X-ray spectroscopy of the deposits of *Porcellio scaber* revealed that the deposits are fully electron and X-ray amorphous (Ziegler, A. 1994; Becker, Bismayer et al. 2003). Light microscopical and ultrastructural analysis has shown that the fully developed sternal CaCO$_3$ deposits of *P. scaber* consist of an opaque region next to the old cuticle composed of numerous microspherules and a glassy layer of homogeneous appearance (hl) adjacent to the sternal epithelium (Figure 1.1b, c) (Ziegler, A. 1994). The spherular region is further subdivided into a distal spherular layer (dsl) consisting of spherules with diameters of 500 nm that are mostly fused (Figure 1.1d) and a proximal spherular layer (psl) of free spherules (Figure 1.1b, c) with diameters ranging from 500 nm in the distal to about 2 µm in the proximal regions (Ziegler et al. 1997). The deposits contain an organic matrix organized in radial and concentric structures which seem to serve as a template for the structural organization of the various layers and which must stabilize the amorphous state of the deposits (Ziegler, A. 1994).

Fabritius et al (2004) reveals that the ultrastructure of the organic matrix is similar within each of the three layers of the CaCO$_3$ deposits. Together with previous investigations on deposit composition (Ziegler, A. 1994; Becker, Bismayer et al. 2003) and structural changes during the molt cycle (Fabritius et al. 2003); the results lead to a better understanding of matrix function. These studies suggest that differences in the solubility of amorphous calcium carbonate within individual spherules are caused by variations in the stabilizing properties of the organic matrix. The structural organization in radial strands and concentric reticules is similar within each layer of the
deposits suggesting that the transition between the three layers is caused by changes in the number of spherule nucleation sites. Such a change in spherule nucleation sites has also lead to the development of two and three layered deposits from primordial single layered deposits during the evolution of terrestrial isopods (Fabritius et al. 2004).

Fabritius et al. (2004) shown that the matrix filaments in the spherules of the distal and the proximal spherular layer (dsl and psl) and in the homogeneous layer (hl) are of the same diameter down to 5 nm. Larger strands of up to 30 nm were also similar in all three layers and are likely to be due to the assembly of additional filaments. These filaments may either be of the same type as the 5 nm filaments or of different types with larger diameters. At the nanoscale the filaments constitute two types of matrix structures (Figure 1.2a-c): reticules that form concentric layers in spherules or spherical calottes in hl, and radial strands, which connect the reticular structures. The arrangement of the reticular and radial structures within the three layers is also very similar. Statistical analysis indicates that the distance between adjacent layers is similar within spherules of dsl and psl. As a consequence, the differences in the size between the spherules result from differences in the number of concentric layers rather than differences in the distance between adjacent concentric layers. Even the spherical calottes in hl, which arise from spherical structures, have distances between one another similar to the distances between the concentric layers in spherules. Together these results make it evident that all three layers of the sternal ACC-deposits arise from the concentric growth of ACC-spherules with striking similarities in the nano-architecture of their organic matrix. The organization of the organic matrix (Fabritius et al. 2004) also provides some evidence for the mechanism of hl formation.
Figure 1.2. Ultrastructure of the organic matrix. Ultrastructure of the organic matrix within the proximal spherular layer (psl). (a) Knife polished sagittal face of spherules treated with Ches buffer pH 10/5 min. Areas around the centre are dissolved leaving radial strands. (b) Whole mount decalcified using 0.2M EDTA/60 min. The core material is dissolved leaving hollow spheres and radial strands within the lumen. Sometimes more than one concentric layer is visible (arrows). (c - g) Matrix in knife polished sagittal faces of psl spherules. (c) 0.1M EDTA, cryo/10 min: Typical spherule with six concentric layers (0-5) connected by radial strands. Sample was viewed in a cryo-holder at -130°C. Source: Fabritius et al. 2004.

In hl circular concentric layers in complete spherules are very rare indicating that the rate of nucleation site formation ceases almost completely. This results in deposition of ACC/matrix material to the proximal spherical surfaces of the last row of polyhedral shaped structures of the transition zone only. The broadening and narrowing of spherical sectors apparent in knife polished and a decalcified sagittal
face of hl (Figure 1.3c) indicates a competitive growth pattern of ACC/Matrix material.

![Figure 1.3](image)

**Figure 1.3. Competitive growth pattern.** Competitive growth pattern of hl segments originating from psl spherules. Source: Fabritius *et al.* 2004.

The molecular construction of these architectures is based on the balancing of hydrophobic-hydrophilic interactions that exist for amphiphilic molecules in aqueous environments, and in the absence of external scaffolds generally adopt a spherical morphology. Many shells and teeth are constructed within frameworks that may be features of the assembly (Weiner *et al.* 1986). First, a relatively inert structural frame is built from insoluble macromolecules such as hydrophobic proteins and/or polysaccharides (chitin). Second, acidic proteins rich in aspartic acid, and often in association with sulfated polysaccharides, are assembled on the hydrophobic scaffold. The onset of mineralization then takes place at the interface between the acidic proteins and aqueous environment.
1.2 Controlled nucleation of biological minerals

The fabrication of biogenic minerals involves the controlled nucleation of inorganic clusters from aqueous solution. In this process, the first-order molecular construction of organic supramolecular systems, (e.g., ferritin micelles, polymeric networks) provide a framework for the second-order assembly of the inorganic phase. These preorganized architectures consist of functionalized surfaces that serve as blueprints for site-directed inorganic nucleation (Figure 1.4) (Mann, S. 1996).

![Figure 1.4. Spatial charge distributions of functional groups.](image)

Spatial charge distributions of functional groups on organic blueprints that differ in topography: (a) concave, (b) convex, and (c) planar. Concave localities give rise to a significant three-dimensional clustering of ionic charge (solid and open circles). Planar substrates can generate periodic arrangements of functional groups. Source: Mann, S. Biomimetic Materials Chemistry, VCH Publishers, Inc., 1996, 15.

Although specific details are not yet available, it is generally considered that the assembly of mineral nuclei is governed by electrostatic, structural, and sterochemical complementarities at the inorganic-organic interface (Mann, S. 1998). This aspect of molecular tectonics, in which interfacial molecular recognition facilitates the construction of nuclei, often with specific crystallographic structures and orientation, is not only a central feature of controlled
biomineralization, but has important generic implications in synthetic materials chemistry.

The role of an organic matrix, such as collagen or ferritin, to be analogous to that of an enzyme in solution, with the incipient inorganic nucleus as the corresponding substrate. But the long-range electrostatic forces of ionic surfaces and the requirement of space symmetry indicates that factors such as lattice geometry, spatial charge distribution, hydration, defect states, and surface relaxation need to be considered along with the sterochemical requirements of ion binding at the interface. A description of the molecular forces operating at the interfaces involving inorganic clusters and macromolecular frameworks is not currently available. Mann, S (1998) state that the role of an organic surface involved in inorganic crystallization is primarily to lower the activation energy of nucleation ($\Delta G^\#$). There is also the possibility of influencing the collision frequencies (preexponential factors of the nucleation rate). For the most simple case,

$$\Delta G^\# = \frac{B \sigma^3 \nu^2}{(kT \ln S)^2}$$

where $B = \text{constant (16} \pi/3 \text{ for a spherical nucleus)}$

$\sigma =$ interfacial energy

$\nu =$ molecular volume

$k =$ Boltzmann’s constant

$T =$ temperature

$S =$ supersaturation

The equation is derived by assuming that nuclei will develop into stable entities only if the energy released through the formation of
bonds in the solid state is greater that that required to maintain the newly created solid-liquid interface. No account is taken of the dependence of $\Delta G^*$ on the two-dimensional structure of different crystal faces or of the effect of extraneous surfaces in the medium. Clearly, both these factors influence the interfacial energy such that there may be an ensemble of nucleation profiles that are crystallographically specific and dependent on the nature of the substrate. Although unconventional, it is useful to consider the nucleation of biominerals in terms of the general ideas of transition state theory, with incipient nuclei of different structure or orientation represented as a series of activated clusters that differ in $\Delta G^*$. Consequently, their steady state concentration and the frequency with which they transform into thermodynamically stable entities will depend on their corresponding reaction trajectories which, in turn, are determined by the specificity of molecular recognition processes. In this way, metastable polymorphs (e.g., vaterite) and specific crystal faces can be preferentially nucleated by the stabilization of particular transition states at the matrix surface (Figure 1.5).

The specific lowering of the activation energy of nucleation reflects a requirement for structural and stereochemical complementarities between the inorganic and organic surfaces. Coordination environments in the mineral phase can be simulated by metal-ion binding to appropriate ligands exposed at the organic surface. Carbonate biomineral tend to be associated with carboxylate-rich (aspartate, glutamate) proteins (Weiner et al. 1986). In this case, the organic residues can mimic the oxyanion stereochemistry of particular crystal faces, and this may be sufficient to induce oriented nucleation.
Similarly, biological deposition of silica is associated with hydroxyl-rich macromolecules rich proteins (Swift et al. 1992).

**Figure 1.5. Diagrammatic representation of the activation energies.**

Diagrammatic representation of the activation energies of nucleation, $\Delta G^\#$, of inorganic minerals in the absence (state 1) and presence (state 2) of an organic surface involved in biomineralization. Three possibilities exist for a mineral of two polymorphic structures (or two nucleation orientations), A and B, where A is the more kinetically favored in the absence of the organic matrix. (a) Nonspecific nucleation catalysis in which both polymorphs (and crystal faces) have reduced activation energies because of the presence of the matrix surface but there is no change in the outcome of mineralization. (b) Structure-specific nucleation of polymorph (or crystal face) B due to molecular recognition and high fidelity synthesis or replication of the matrix surface. (c) Combination of (a) and (b), depending on the levels of recognition of nuclei A and B and the fidelity of matrix production, both of which may be influenced by genetic, metabolic, and environmental processes. Source: Mann, S. Biomimetic Materials Chemistry, VCH Publishers, Inc., 1996, 15.

1.2.1 Oriented nucleation induced by Organic Surfaces

In extended periodic structures (assemblies) such as antiparallel $\beta$-pleated sheets (shells), $\alpha$-helices (fish antifreeze proteins), polysaccharide matrices, and proteolipid and phospholipids membranes can control the assembly of nuclei along specific crystallographic directions. In this respect, the secondary, tertiary, and
quaternary conformations of macromolecules are key features of the blueprint required for controlled nucleation. In such cases, the long-range translational symmetry of inorganic lattices is established at the surface of the organized organic surface by regulating the spatial disposition of functional groups across the matrix surface (Figure 1.6).

**Figure 1.6. Epitaxy in biomineralization.** Geometric matching (epitaxy) in biomineralization. Cation-cation distances in one specific crystal face are commensurate with the spacing of periodic binding sites on an organic surface. Source: Mann, S. Biomimetic Materials Chemistry, VCH Publishers, Inc., 1996.

In invertebrates, electron diffraction studies of partially demineralized mollusk shells have shown that in some species both the \( a \) and \( b \) axes of an antiparallel \( \beta \)-pleated sheet protein are aligned with the \( a \) and \( b \) crystallographic directions of aragonite (\( \text{CaCO}_3 \)) (Weiner *et al.* 1984). Partial amino acid sequencing of these acidic proteins (Rusenko *et al.* 1991) has indicated that there are repeated domains of polyaspartate that could be the nucleation centers. In general, the evidence is mounting that surface binding motifs involving blocks of sequences such as \([\text{Asp}]_n\) and \([\text{PSer}]_n\) are common throughout biomineralization. One possibility to regulate oriented nucleation is
that there is geometric matching (epitaxy) between the lattice spacing of ions in crystal faces and functional groups arranged across the organic surface. For example, the distances between aspartic acid residues deployed along a $\beta$-pleated sheet are similar to the Ca-Ca distances in the nucleated aragonite (001) face observed in the mollusk shell (Weiner et al. 1984). Because the binding constants for Ca at carboxylate sites are not high, it has been suggested that nucleation is a cooperative process involving structurally disordered sulfate groups of flexible oligosaccharide side chains and organized motifs of carboxylate ligands within $\beta$-pleated sheet surface domains of the matrix (Addadi et al. 1992). The former provides a flux of Ca to the nucleation site, while the latter induces oriented nucleation. These suggestions are borne out by model systems in which calcite crystals were grown on sulfonated polystyrene films with or without adsorbed polyaspartic acid. (Addadi et al. 1987) Rigid, highly sulfonated films induce the preferential nucleation of the calcite (00.1) face, and this is increased tenfold in the presence of adsorbed polyaspartate in the $\beta$-pleated sheet conformation. Adsorption of polyglutamate, which mainly adopts a random conformation, does not show this effect (Mann et al. 1996).

1.2.2 Shaping of Biogenic minerals

The shaping of individual biominerals is often accompanied by their assembly into organized ultrastructures. Construction of these higher order architectures within the cellular space can be based on either static or dynamic principles. An example of an organizational motif that is relatively rigid and spatially fixed is the linear assemblies of a chain of membrane-bound calcite crystals are organized in radial arrangements of vesicles to produce coccolith scales in marine alga.
this system, the individual scales are exocytosed and assembled into a coccosphere that surrounds the cell wall of the alga (Figure 1.7).

![Intact coccosphere of *E. huxleyi*](image)

**Figure 1.7.** Intact coccosphere of *E. huxleyi*. Intact coccosphere of *E. huxleyi* showing the assembly of coccoliths against the cell wall. Source: Henriksen et al. American Mineralogist, 2003, 88, 2040.

Macroscopic organized architectures such as bone, shells, and teeth, are associated with extended extracellular matrices, and their hierarchical structure produces composite materials that in the case of bone are capable of being remodeled by active cellular processing in response to mechanical stress. Another feature of these systems is slow repetitive processing. For example, the organized architecture of cuttlefish bone is constructed from the episodic deposition and processing of an extracellular polymeric (chitin) matrix (Birchall et al. 1983). Similarly, in the nacreous layer of shells, sheet-like organic assemblies are secreted periodically beyond the mineralization front, leading to progressive infilling that result in the construction of a highly organized lamellar architecture (Bevelander et al. 1969). While
the details of the recognition and organizational processes involved in the construction of these higher order biomineralized architectures are currently unknown, it is probable that the phenomenon is a consequence of what one might describe as cellular logistics (Mann et al. 1996). In particular, in unicellular organisms such as coccoliths and choanoflagellates, it appears that the organized architectures are generated from the coupling of the (intracellular) construction process, with generic processes involving the movement and organization of materials to and from cell periphery. These processes utilize motive forces such as generated by vesicle trafficking along cytoskeletal frameworks, flagella activity, or cytoplasmic streaming. By contrast, multicellular systems possibly construct higher order mineralized architectures by coupling biomineralization to intercellular communication concerned with the organization of supplies and functions (services) across relatively long length scales.

1.3 Biomimicry

The specific chemical and structural properties of biomolecules involved in biomineralization processes can be utilized in synthetic reactions—for example, shell proteins in the control of the texture and morphology of calcite crystals (Berman et al. 1988). Biomimicry is the use of bioconcepts as sources of inspiration for new ideas in the synthesis of inorganic materials exhibiting uniform particle size, polymorph selectivity, tailored morphology, oriented nucleation, and organized assembly. In essence, the problem is to elucidate strategies that integrate a molecular tectonics perspective of biomineralization with knowledge of inorganic material chemistry. In general, the approaches will be based on the use of preformed supramolecular assemblies in the control and organization of inorganic materials
synthesis, and the application of molecular recognition at organic-inorganic interfaces will be paramount. More specifically, the four biological archetypes described by Mann, S (Mann et al. 1996) can be translated into principles that serve as guidelines for laboratory development (Table 1.1).

<table>
<thead>
<tr>
<th>Biological Archetypes</th>
<th>Biomimetic principle</th>
</tr>
</thead>
<tbody>
<tr>
<td>Supramolecular preorganization</td>
<td>Host-guest material chemistry</td>
</tr>
<tr>
<td>Interfacial molecular recognition</td>
<td>Molecular blueprinting</td>
</tr>
<tr>
<td>Vectorial regulation</td>
<td>Crystal engineering</td>
</tr>
<tr>
<td>Cellular processing</td>
<td>Crystal tectonics</td>
</tr>
</tbody>
</table>


First, the preorganization of supramolecular assemblies in biomineralization can be considered as an archetype of a host-guest approach to the nanoscale synthesis of composites. Second, the concept of interfacial molecular recognition suggests that inorganic materials synthesis can be regulated through molecular blueprinting with organic substrates. Third, the crystal engineering of materials can be viewed from the perspective of vectorial regulation with concomitant concepts such as templating, directed growth, and microstructural fabrication. Finally, the paradigm of cellular processing of biominerals can be used to develop methods to the controlled architectural
assembly of preformed particles or crystals with tailored properties—an approach that extends all the way from molecular to crystal tectonics.

1.4 Theory

In general, precipitation or reaction crystallization is considered to be a particular form of crystallization. A distinct feature lies in the fact that compared to other forms of crystallization the solubility of the crystallizing substances is vastly exceeded. An example is the mixing of two solutions of calcium chloride and sodium carbonate. Calcium carbonate forms instantaneously and precipitates due to its low solubility. The product particle size obtained in such a precipitation can be considerably smaller compared to other crystallization processes that operate closer to the solubility of the substance. These and other aspects of precipitation can be explained by considering some fundamental principles addressed in the following.

1.4.1 Equilibrium and Supersaturation

In precipitation a non-equilibrium state is imposed onto a system. This must be seen in relation to an equilibrium state of the system. To be able to describe the state of a system a differential form of the fundamental equation of thermodynamics is usually employed. The internal energy of a system is a function of its entropy, volume and mole numbers of its components (Stumm et al. 1996).

\[
dU = TdS - pdV + \sum_i \mu_i dn_i
\]  

(1)

The form of the equation indicates that whereas temperature is responsible for heat transfer and pressure for volume work, the chemical potential controls mass transfer (Stumm, W.; Morgan, J. J.)
The definition of Gibbs free energy is given through (Atkins et al. 1994, Stumm, W.; Morgan, J. J. 1996):

\[ G = H - TS = U + pV - TS \]  

(2)

The differential form can then be written as:

\[ dG = dU + pdV + Vdp - TdS - SdT \]  

(3)

The differential form of the fundamental thermodynamic Equation (1) is introduced which renders:

\[ dG = \sum \mu_i dn_i + Vdp - SdT \]  

(4)

A distinct feature of reaction crystallization lies in the fact that it is initiated by embossing an unstable state onto a system. The system reacts by striving towards equilibrium. This equilibrium is characterized by a minimum of the Gibbs free energy of the system, i.e. (Atkins 1994, Stumm, W.; Morgan, J. J. 1996):

\[ dG = 0 \]  

(5)

Furthermore, precipitation is thought to occur at a fixed temperature and pressure and therefore Equation (4) may be simplified to give (Stumm, W.; Morgan, J. J. 1996):

\[ dG = \sum \mu_i dn_i \]  

(6)

Having to use Gibbs free energy directly in the treatment of chemical equilibria would be quite complicated. It can be made simpler which is described in the following. A chemical reaction produces a change in the quantity of a species. \( \nu_i \) is the stoichiometric coefficient of a species \( i \) and \( \xi \) is the advancement variable of the reaction (Stumm, W.; Morgan, J. J. 1996). This is then introduced into equation (6).
\[ dn = v d \xi \]  

(7)

\[ dG = \sum_i v_i \mu_i d \xi = \Delta G d \xi \]  

(8)

The expression \( \Delta G \) is commonly referred to as the Gibbs free energy change of the reaction (Stumm, W.; Morgan, J. J. 1996). At equilibrium the free energy of change also becomes “0”.

\[ \Delta G = \sum_i v_i \mu_i \]  

(9)

\[ dG = 0 \leftrightarrow \Delta G = 0 \]  

(10)

It is now necessary to express the chemical potential of a species. This is commonly done using its activity (Stumm, W.; Morgan, J. J. 1996).

\[ \mu = \mu_i^0 + RT \ln a_i \]  

(11)

From the perspective of physics the activity should correctly be expressed in terms of mole fractions. However due to practical reasons concentration, partial pressure and molality are often used instead. When calculating the chemical potential it must be considered that electrolytes dissociate (Stumm, W.; Morgan, J. J. 1996)

\[ v \cdot X + v \cdot Y \rightarrow X_v \cdot Y_v \]  

The individual chemical potential of one component is therefore expressed by:

\[ \mu_- = \mu_{i-}^0 + RT \ln a_{i-} \]  

(12)

\[ \mu_+ = \mu_{i+}^0 + RT \ln a_{i+} \]  

(13)

These two expressions (Equation (12) and (13)) may be combined to give:
The activity of a species can be calculated, if the activity coefficients are known. In this case the activity is described in terms of concentration (Stumm, W.; Morgan, J. J. 1996):

\[ \alpha_i = \gamma c_i \]  

(15)

In aqueous solutions the activity coefficients have been shown to depend on the ionic strength. The ionic strength is calculated using the charge of the ionic species \( z \) and their concentrations \( c \).

\[ I = 0.5 \sum_i c_i z_i^2 \]  

(16)

Several expressions have been suggested to express the dependence of the activity coefficient on the ionic strength. Let us use the Davies equation. It is suggested to be valid up to ionic strengths of 0.5 mol l\(^{-1}\) (Stumm, W.; Morgan, J. J. 1996).

\[ \log \gamma = -A z^2 \left( \frac{\sqrt{I}}{1 + \sqrt{I}} - 0.3I \right) \]  

(17)

A is a temperature property of water. At 25°C A is approximately 0.5. The expression for the chemical potential (Equation (11)) is now inserted into Equation (9).

\[ \Delta G = \sum_j v_j \mu_j = \sum_j v_j \mu_j^0 + RT \sum_i v_i \ln a_i = \Delta G^0 + RT \sum_i v_i \ln a_i \]  

(18)

Thus the standard Gibbs free energy change of the reaction is obtained (Stumm, W.; Morgan, J. J. 1996).

\[ \Delta G^0 = \sum_j v_j \mu_j^0 \]  

(19)
The concept of equilibrium constants \(^1\) is now introduced (Stumm, W.; Morgan, J. J. 1996):

\[
\ln K = -\frac{\Delta G^0}{RT} = -\sum_i \frac{v_i \mu_i^0}{RT} \quad (20)
\]

Combining Equation (9) and (20) renders a new expression including the equilibrium constant (Stumm, W.; Morgan, J. J. 1996).

\[
dG = \Delta G = 0 \Rightarrow K = \prod_i a_i^v \quad (21)
\]

To be able to determine whether a solution is super- or undersaturated with respect to a solid phase a new equilibrium constant referred to as the solubility product \(^2\) \(K_{sp}\) is introduced. The Gibbs free energy of dissolution of the solid phase is then given through:

\[
\Delta G = \Delta G^0 + RT \sum_i v_i \ln a_i = RT \left( \sum_i v_i \ln a_i - \ln K_{sp} \right) \quad (22)
\]

If the Gibbs free energy of dissolution is greater than “0” the solution is supersaturated (Stumm, W.; Morgan, J. J. 1996).

\[
\Delta G > 0 \quad (23)
\]

If these two expressions are combined an expression referred to as supersaturation \(S\) can be obtained. The supersaturation allows an estimate of the overall driving force for a system to attain an equilibrium using the chemical potential (Mullin et al. 1993)

---

\(^1\) Equilibrium constants are known for wide variety reactions in various media. For instance the equilibrium constant for the self-dissociation of water is \(10^{13.998}\) at 25\(^\circ\)C.

\(^2\) From a physical perspective solubility products can only be defined for solid phase that is thermodynamically stable on an indefinite time-scale. Yet, solubility products of unstable solid phases are often used to appreciate the conditions that prevail in kinetically stable systems.
\[ S = \prod_{i} \frac{a_i^n}{Ksp} \] (24)

If the supersaturation is higher than 1, the Gibbs free energy of dissolution is greater than 0 and the solution is supersaturated with respect to the solid phase. However, it must be noted that defining supersaturation in this way suggests that for a supersaturation greater than 1, the system should spontaneously equilibrate. This is, however, generally only the case when the solid phase is already present. In absence of a solid phase certain energy must be exceeded for the system to equilibrate spontaneously.

Equilibrium constants generally show a dependency on temperature. To account for this phenomenon the van’t Hoff equation is commonly used in cases where the heat of formation is known.

\[ \frac{d \ln K}{dT} = \frac{\Delta H}{RT^2} \Rightarrow \ln \frac{K_2}{K_1} = \frac{\Delta H}{R} \left( \frac{1}{T_1} - \frac{1}{T_2} \right) \] (25)

The van’t Hoff equation does however not account for any changes in heat capacity. Therefore in some cases where more detailed data is available a more exact semi-empirical equation is used to describe the temperature dependency of the equilibrium constants (Stumm, W.; Morgan, J. J. 1996).

\[ \ln K = C_1 + C_2 T + \frac{C_3}{T} + C_4 \log T \] (26)

1.4.2 Ostwald-Freundlich relation

The relationship (24) derived above does not consider the form or shape of the solid phase to influence the equilibrium state of a system. It can, however, be shown that the ratio area versus volume of
a solid phase plays an important role in terms of solubility. Lord Kelvin (formerly Thomson) originally derived such a relationship for vapor pressures in liquid-vapor systems. Ostwald and Freundlich later applied the relationship to solid-liquid systems. Therefore it is commonly referred to as the Ostwald-Freundlich relation. It has been thermodynamically derived elsewhere. Since it appears in a number of forms, it is once again derived here for conditions that apply to this work (Wu et al. 1999)

The change in Gibbs free energy of a particle in quantity of molecules/ions $dn$ or a change in surface area, using the interfacial energy, can be expressed by:

$$dG = \mu^0 dn + \sigma dA$$  \hspace{1cm} (27)

The chemical potential is defined through:

$$\mu_i = \left( \frac{\partial G}{\partial n_i} \right)_{p,T,n_j}$$  \hspace{1cm} (28)

Assuming isothermal and isobaric conditions this renders:

$$\frac{dG}{dn} = \mu = \mu^0 + \sigma \frac{dA}{dn} = \mu^0 + \sigma \frac{M}{\rho} \frac{dA}{dV}$$  \hspace{1cm} (29)

Under the assumption that the area and volume both can be attributed to a characteristic length $L$, an expression to substitute $dA/dV$ is derived.

$$A = K_A L^2 \Rightarrow dA = 2K_A L dL$$  \hspace{1cm} (30)

$$V = K_V L^3 \Rightarrow dV = 3K_V L^2 dL$$  \hspace{1cm} (31)

$$\frac{dA}{dV} = \frac{2K_A L}{3K_V L^2} = \frac{2A}{3V}$$  \hspace{1cm} (32)
Inserting into Equation (30) renders:
\[
\mu = \mu^0 + \frac{2M\sigma A}{3\rho V}
\]  
(33)

An expression for the chemical potential has been derived previously (Equation 14). Under equilibrium conditions the standard chemical potential of a particle of infinitely large characteristic length is assumed to correspond to the chemical potential of a saturated solution. This implies that the solubility product is defined for a flat surface with an infinitely large characteristic length.

\[
\lim_{L \to \infty} \frac{A(L)}{V(L)} = \lim_{L \to \infty} \frac{K_d L^2}{K_y L} = \lim_{L \to \infty} \frac{K_d}{K_y L} = 0
\]  
(34)

\[
\mu^0 = \mu^0_{\text{eq}} + RT \ln a_{i-} \left( \frac{A}{V} \to 0 \right) a_{i+}^{v+} \left( \frac{A}{V} \to 0 \right) = \mu^0_{\text{eq}} + RT \ln \text{Ksp}
\]  
(35)

Under equilibrium conditions the chemical potential of the particle equals the chemical potential of the solution.

\[
\mu^0_{i-+} + RT \ln a_{i-} \left( \frac{A}{V} \to 0 \right) a_{i+}^{v+} \left( \frac{A}{V} \to 0 \right) + \frac{2M\sigma A}{3\rho V} = \mu^0_{i-+} + RT \ln a_{i-}^{v-} a_{i+}^{v+}
\]  
(36)

\[
\Rightarrow \ln \frac{a_{i-}^{v-} a_{i+}^{v+}}{a_{i-}^{v-} \left( \frac{A}{V} \to 0 \right) a_{i+}^{v+} \left( \frac{A}{V} \to 0 \right)} = \ln \frac{a_{i-}^{v-} a_{i+}^{v+}}{\text{Ksp}} = \ln S = \frac{2M\sigma A}{3RT\rho V}
\]  
(37)

A commonly made assumption is that the particle is spherical in shape.

\[
\frac{A}{V} = \frac{4\pi r^2}{3} = \frac{3}{4\pi r^3}
\]  
(38)
By introducing the Boltzmann constant \( k \) and the molecular volume \( V_m \) Equation (37) may be further modified.

\[
k = \frac{R}{N} = 1.3805 \times 10^{-23} \text{JK}^{-1}
\]

(39)

\[
V_m = \frac{V}{nN} = \frac{M}{N\rho}
\]

(40)

\[
\Rightarrow \ln \frac{a_i^{r-}a_i^{r+}}{a_i^{r-}(r \to \infty)a_i^{r+}(r \to \infty)} = \ln \frac{a_i^{r-}a_i^{r+}}{Ksp} = \ln S = \frac{2\sigma V_m}{kTr}
\]

(41)

This is the Ostwald-Freundlich relation in the form used in this work. It gives the effective supersaturation for a solution in equilibrium with particles of a certain size, i.e. particle radius. The relation is illustrated for the particular case of the calcium carbonate polymorph calcite in Figure 1.8.

Figure 1.8. Ostwald-Freundlich relation. Effective supersaturation for a solution in equilibrium with particles of a certain size, i.e. particle radius, calculated using the Ostwald-Freundlich relation for the case of calcite. Source: Wu et al. 1999.
1.4.3 Nucleation

The formation of a new solid phase from a liquid is initiated through a mechanism referred to as nucleation. Nucleation is commonly divided into nucleation without the involvement of crystals in the formation of a solid phase, referred to as primary nucleation and nucleation by mechanisms that require the presence of crystals of the forming solid phase which is referred to as secondary nucleation (see Figure 1.9).

![Nucleation Diagram](image)

**Figure 1.9. The different forms of Nucleation.** The different forms of nucleation. Source: Mullin, J. W. Crystallization, 1993, Oxford: Butterworth Heinemann Ltd.

If the crystal nuclei are formed without the involvement of any foreign matter, it is referred to as homogeneous primary nucleation. If foreign matter, e.g. dust particles, in the system contributes to the formation of nuclei, it is referred to as heterogeneous primary nucleation (Mullin *et al.* 1993, Mersmann *et al.* 1995). In the following, the classical nucleation theory describing the rate of homogeneous nucleation and its dependence on the supersaturation is derived. It is based in the Ostwald-Freundlich relationship. To begin with the change in Gibbs free energy of a nucleus of radius \( r \) is the sum of its
Gibbs free energy and its Gibbs free volume energy created through the formation of a nucleus of a certain volume. This assumes that the interfacial energy may be defined for a finite surface. Gibbs definition of the interfacial energy relates to a more or less diffuse interface. Furthermore, it assumes that the nuclei are spherical in shape and that a constant interfacial energy may be defined for its entire surface (Zettlemoyer et al. 1969, Mullin et al. 1993, Mersmann et al. 1995)

\[ \Delta G = \Delta G_s + \Delta G_v = 4\pi r^2 \sigma + \frac{4}{3}\pi r^3 \Delta G_v \]  

(42)

The free surface energy is calculated by multiplying the assumed finite nucleus surface with the interfacial energy. \( \Delta G_v \) is the change in Gibbs free energy per unit volume due to the transformation of dissolved matter into solid matter. \( \Delta G_v \) is a negative quantity. The second term is the one forcing the system to reach equilibrium (see Figure 1.10).

![Diagram](image)

**Figure 1.10. Gibbs free energy of a nucleation.** The dependence of the change in Gibbs free energy of a nucleation on its dimension. Source: Mersmann et al.
The maximum value $\Delta G_{\text{crit}}$ corresponds to the critical radius of a nucleus. Any nucleus smaller than the critical size will dissolve from a thermodynamic point of view. A critical size and a critical Gibbs energy can then be derived.

\[
\frac{d\Delta G}{dr} = \frac{d}{dr} \left( 4\pi r^2 \sigma + \frac{4}{3}\pi r^3 \Delta G_y \right) = 8\pi r \sigma + 4\pi r^2 \Delta G_y
\]  

(43)

\[
\Rightarrow r_{\text{crit}} = -\frac{2\sigma}{\Delta G_y} \Rightarrow \Delta G_{\text{crit}} = \frac{4\pi \sigma r_{\text{crit}}^2}{3}
\]  

(44)

At this point the Ostwald-Freundlich relationship for an electrolyte is introduced. The Ostwald-Freundlich relation correlates the solubility of particle to its size. Thus with the aid of the Ostwald-Freundlich relation it can be determined, whether a nuclei is thermodynamically stable, or not (Zettlemoyer et al. 1969, Mullin et al. 1993).

\[
r_{\text{crit}} = \frac{2\sigma V_m}{kT \ln S}
\]  

(45)

Above this critical radius, a particle is thermodynamically stable. The previous results from Equation (44) are inserted.

\[
\Rightarrow \Delta G_{\text{crit}} = \frac{16\pi \sigma^3 V_m^2}{3(kT \ln S)^2}
\]  

(46)

A critical Gibbs free energy has been derived. It has been proposed that the kinetics of homogeneous nucleation can be described by an expression of the following form (Nielsen et al. 1964).

\[
J_{\text{hom}} = A_{\text{hom}} \exp \left( -\frac{\Delta G_{\text{crit}}}{kT} \right)
\]  

(47)
This equation is similar to a 0\textsuperscript{th} order reaction. Strictly speaking, however, nucleation cannot be considered a chemical reaction of a certain order. The previous results are inserted (Nielsen et al. 1964).

\begin{equation}
J_{\text{hom}} = A_{\text{hom}} \exp \left( -\frac{16\pi\sigma^2v_m^2}{3(kT)^3(\ln S)^2} \right)
\end{equation}

This equation describes the homogeneous nucleation rate in a supersaturated solution. Four main parameters influence the nucleation rate, i.e. the interfacial energy, the temperature, the supersaturation and the pre-exponential factor. Zettlemoyer (Zettlemoyer et al. 1969) suggests that the pre-exponential factor is lowered by a lower energy barrier to desolvation and blocking tendencies of the escaping solvent molecules. The equation indicates that the supersaturation controls the rate of nucleation significantly (see Figure 1.11).

\begin{figure}[h]
\centering
\includegraphics[width=0.5\textwidth]{figure11.png}
\caption{Rate of nucleation. Dimensionless nucleation rate, i.e. $J_{\text{hom}} / A_{\text{hom}}$, versus supersaturation for the case of calcite at 50\textdegree C. The homogeneous nucleation rate starts to increase dramatically above a supersaturation of 5. Source: Zettlemoyer et al. 1969.}
\end{figure}
Figure 1.11 predicts phenomena generally observed. Unless a solid phase is already present, a certain critical supersaturation significantly greater than 1 needs to be exceeded for the system to equilibrate spontaneously by nucleation. The nucleation equation derived is able to describe this behavior within certain limits. The supersaturation zone between saturation and the critical supersaturation where homogeneous nucleation occurs is commonly referred to as the metastable zone (see Figure 1.12).

**Figure 1.12. Supersaturation Zone.** In the zone of dissolution below supersaturation the solid phase will dissolve. In the metastable zone below a certain critical supersaturation crystal growth and secondary nucleation can be observed. Both these phenomena are related to the existence of a solid phase. In the unstable zone a solid phase may form spontaneously without the involvement of the solid phase. This phenomenon is called primary nucleation. Source: Mullin et al. 1993. The width of the zone, i.e. the critical supersaturation, may vary significantly depending on the conditions. Within the metastable zone supersaturation can only be consumed by crystal growth and so-called
secondary nucleation onto an already existing solid phase (Mullin et al. 1993).

It is generally assumed that heterogeneous nucleation always occurs in parallel to homogenous nucleation. However, its role diminishes with increasing supersaturation. Heterogeneous nucleation occurs, since it is virtually impossible to produce a solution without traces of foreign matter. The rate of nucleation can be substantially influenced by even trace amounts of impurities in the system. These may act as inhibitors, as well as accelerators for nucleation. Foreign particles may have a catalytic effect, thus decreasing the energy barrier, if the nuclei form on the surface of foreign matter. Therefore, it may appear as though the width of the metastable zone is increased occurring on foreign particles; a correction factor $f$ has been suggested (Mullin et al. 1993, Mersmann et al. 1995, Söhnel et al. 1992).

$$J_{het} = A_{het} \exp \left( -f \frac{16 \pi \sigma^2 v^2}{3(kT)^2 (\ln S)^2} \right) \quad (49)$$

The factor is given by a relationship that includes the wetting angle (Mersmann 1995):

$$f = \frac{(2 + \cos \Theta)(1 - \cos \Theta)}{4} \quad (50)$$

The correction factor ranges from 0 to 1. The effect that it has on the nucleation rate is shown in Figure 1.13 below.
As previously mentioned, secondary nucleation occurs within the metastable zone. Several potential mechanisms have been proposed (Randolph et al. 1988, Söhnel et al. 1992, Mullin et al. 1993)

- Nuclei formation by attrition and fracture following crystal-crystal or crystal-apparatus interactions.
- Needle structures on the surface such as dendrites that continuously break off to form nuclei.
- Nuclei forming as molecular aggregates with a crystalline structure in the adsorption layer on the surface of the growing crystal are washed into the solution especially at high fluid shear rates.
- Contact nucleation where nuclei are formed by collisions between crystals or between crystals and apparatus causing a displacement of the adsorbed although not yet crystalline layer of solute.
Formation of nuclei in the liquid phase due to structural changes of the liquid adjacent to a crystal or as a result of the presence of dissolved admixtures inhibiting the crystallization process.

To study nucleation is due to fast kinetics quite challenging from an experimental point of view. The induction time of a precipitation process may be used to study nucleation. Generally the induction time of primary nucleation is thought to consist of three distinguishable time intervals: the initial transient period, the nucleation time and the growth time.

\[ t_{\text{ind}} = t_{\text{tr}} + t_{\text{n}} + t_{\text{g}} \quad (51) \]

Assuming the nucleation time to be much longer than the transient time as well as the growth time, the following relation may be established between the nucleation rate and the induction time for the case of steady state nucleation:

\[ J = \frac{N}{t_{\text{ind}}} \Rightarrow N = Jt_{\text{ind}} \quad (52) \]

N corresponds to particle concentration. Under the assumption that the induction time is observed for the same particle concentration, a plot of supersaturation versus induction time can be used to obtain the interfacial energy (Wu et al. 1999). Commonly this plot is performed in the form of \( \ln t \) versus \( \ln^2 S \) and thus the interfacial energy can be obtained from the slope of a liner regression. Kashcheiv

---

3 Induction time can be taken as the time interval needed for a supersaturated solution to produce a detectable solid phase. Detection can be performed by any means thought to produce a reproducible result.
and co-workers (Kashchiev et al. 1991) have developed a method to account for a contribution of growth time to the induction time. Yet, to be applied this method requires a certain degree of knowledge of the crystal growth mechanisms in the form of a growth equation. Kashcheiv and Sato (Kashcheiv et al. 1998) have also developed a theoretical description of the kinetics of overall crystallization for the case of nucleation of a stable phase being preceded by the formation of a metastable phase. It must also be that a significant number of substance exhibit polymorphism4. The interfacial energy and molecular volume may vary from one polymorph to another. Since polymorphs can form simultaneously during an experiment, experimentally obtained interfacial energies must often be considered as being intermediate values. Furthermore, a substantial amount of nuclei may form through heterogeneous nucleation. There is some evidence or a critical supersaturation, above which homogeneous tends to dominate. Several authors therefore distinguish between the interfacial energy of homogeneous and heterogeneous nucleation (Mullin et al. 1993).

1.4.4 Crystal Growth

A supersaturated solution containing stable crystal surfaces induces crystal growth on these surfaces. The consumption of supersaturation is governed by the growth rate of the growing surfaces and the overall surface area. Generally, the overall growth rate \( G \) is defined for a characteristic length \( L \) of the crystal (Mullin 1993).

\[
G = \frac{dL}{dt}
\]

(53)

---

4 Polymorph – “Poly” many, “morph” shape. Solid substance may occur in different solid crystalline forms. A famous example is carbon having the solid form of graphite and diamond. In case of calcium carbonate, polymorphs incorporate hydrated forms of calcium carbonate. The true polymorph designates only anhydrous forms, i.e. CaCO₃.
For a molecule or an ion to be integrated into the crystal lattice several barriers must be overcome. These are in order of their suggested appearance: diffusion through the boundary layer surrounding the crystal, adsorption onto the crystal surface, surface diffusion to a growth site and integration into the crystal lattice. When studying crystal growth, it is often hard to determine the exact mechanisms of growth. Therefore the overall growth rate is often correlated using simple empirical power laws with supersaturation as a driving force (Mullin 1993):

\[ G = K_g (S - 1)^n \]  

(54)

Still, a more detailed analysis of the crystal growth process is possible. The mechanism involved in crystal growth of ionic crystal can be quite complicated. There is a possibility of an intermediary dissolved component forming at any point during growth. Different mechanisms for ionic crystal growth have therefore been suggested. Some of these mechanisms are illustrated in Figure 1.14 to 1.17 (Mersmann 1995).

**Figure 1.14. Ionic crystal growth-I.** The dissolved ions are individually adsorbed. The intermediary component is formed the adsorption layer and is then integrated into the crystal lattice. Source: Mersmann 1995.
Figure 1.15. Ionic crystal growth-II. The dissolved ions are adsorbed and integrated into the crystal lattice individually. Source: Mersmann 1995.

Figure 1.16. Ionic crystal growth-III. The dissolved ions form an intermediary component while dissolved. The component diffuses through the boundary layer and is directly incorporated into the crystal lattice. Source: Mersmann 1995.

Figure 1.17. Ionic crystal growth-IV. The dissolved ions form an intermediary component within the boundary layer. The component is adsorbed and incorporated into the crystal lattice. Source: Mersmann 1995.
Crystal growth can be considered to occur through all of the shown mechanisms. The rate of a crystal growth mechanism is limited by its slowest step. The crystal growth rate can e.g. be diffusion controlled, if the diffusion rate is far lower than the surface integration rate.

Over the years, a number of more sophisticated models to describe crystal growth have been suggested. Some models assume two-dimensional nucleation to be necessary for layer growth, making them hard to apply at lower supersaturations. This is due to the fact that solutions only nucleate at very low rates at low supersaturations. Consequently the model cannot predict the production of a sufficient number of growth sites. The Burton-Cabrera-Frank (BCF) model overcomes this problem by suggesting that screw dislocations are the source of new steps. In the form of the BCF surface diffusion model, it considers crystal surface diffusion to be the rate limiting step. At low supersaturation the model predicts the growth rate to be proportional to the supersaturation to the second power and at higher supersaturations a linear relationship is predicted. Other forms of the BCF model assume bulk diffusion to be the rate-limiting step (Myerson 1993).

1.4.5 Secondary Changes

Certain polymorphs or intermediary forms of a precipitated substance may be thermodynamically unfavorable, but will primarily form when, for instance, supersaturation is introduced rapidly. Some of these phases can be amorphous and consequently hard to characterize. An initially formed phase may simply be the kinetically

---

5 Amorphous implies formless, without shape. Statistically the inner structure is similar in all directions. An example is glass- a sub-cooled liquid.
and not the thermodynamically most favorable state. In the following the solid phase can undergo changes, thus transforming into a thermodynamically more favorable state. The opposite may also be the case and the system may remain in its thermodynamically unfavorable state. Gösele et al. have shown that increasing the supersaturation decreases the possibility of an intermediate phase transforming into a stable phase (see Figure 18, Gösele et al. 1990, Gösele 1993).

**Figure 1.18. Phase transformation.** The resistance towards phase change into a thermodynamically more favorable state may increase with supersaturation. Source: Gösele et al. 1990.

Some of the processes involved in secondary changes of the solid phase are described in the following (Söhnel and Garside 1992, Mullin 1993):

**Ageing** is characterized by slow microscopic and sometimes macroscopic changes of the solid phase properties, such as crystal modification, habit, specific surface, size, or chemical composition of the solid phase. An example of ageing is recrystallization of non-equilibrium shapes of primary particles such as dendrites, needle, or plates to form more compact shapes.

**Aggregation** involves clustering of separate particles to form larger particles. The process can be divided into agglomeration and
coagulation. The first term describes clustering of initially formed particles to form secondary particles held together mainly by crystalline bridges. Coagulation occurs when small particles form clusters that are bound only by physical forces. Agglomeration frequently plays a significant role in particle formation.

**Recrystallization** describes the alteration in size and shape of the crystals through surface integration or mass transfer. Recrystallization can be either isothermal or non-isothermal.

**Isothermal recrystallization (ripening or Ostwald ripening)** shift the crystal size distribution towards larger sizes. The large crystals grow while the small crystal dissolve and successively disappear. The driving force for Ostwald ripening can be explained by the Gibbs-Thomson relationship.

### 1.4.6 Influence of Additives

Generally there are four ways by which an additive can interfere with a crystallization process (see e.g. Raistrick 1949, McCartney and Alexander 1958, van der Leeden et al. 1993):

- An additive may alter the equilibrium state of a system.
- An additive may interfere with nucleation.
- An additive may interfere with crystal growth.
- An additive may interfere with secondary changes.

Changing the equilibrium state of a system may, for instance, occur through complexation of one of the ionic species that a salt consists of. These effects can usually be accounted for in an

---

6 The term additive designates substance not needed for a crystallization process, but with the potential of interfering with it. An additive may speed up or slow down the kinetics of a crystallization process. Impurity is also a term quite often used.
equilibrium model provided the equilibrium constants of the corresponding reaction are known.

The additives may prevent the formation of certain molecular cluster that precede the formation of nuclei or may influence nucleation through adsorption onto the nuclei. It is commonly assumed that homogeneous nucleation is preceded by the formation of solid-like molecular clusters. This may be of relevance, since additives have the capability of forming complexes. These complexes may influence the formation of pre-nuclei clusters and thus the molecular state of the liquid. Packter and Saunders (Packter and Saunders 1970), for instance, explained some of their experimental observations as being the result of additives chemisorbing to the outer regions during nucleation, thus lowering the nucleation rate.

If an additive affects the nucleation process, this should be expressed in terms of change in certain physical properties of the system. According to Liu (Liu 2001) interaction of additives that lead to repulsion and interfacial structure mismatch of the nucleating phase with the nuclei results in an increased energy barrier. The specific calcium complexing carboxylic acids used in this study have the ability to interact with calcium which involves specifically removing it from pre-crystalline clusters and crystal surfaces. If Equation (42) and (46) are considered, it is reasonable to assume that phenomena that counteract the formation of a solid phase lead to an increase in the critical energy. A rise in the critical energy is coupled to an increase in interfacial energy.

A relative nucleation rate may be derived that compares the nucleation rate in the presence of an additive to the rate in the absence of the additive:
Concerning the effect that an adsorbed species may have on crystal growth Cabrera and Vermilyea (Cabrera and Vermilyea 1958) in their model proposed that rigid adsorbed impurities prevent step advancement during layer growth. Only if the distance between adsorbed impurities is larger than a critical distance related to the medium radius of curvature of a step at a given superstauration can a step advance in between.

\[
\frac{V}{V_0} = \sqrt{1 - 2\rho_c d^{0.5}} \tag{56}
\]

\(d\) is the average density of impurities just ahead of the step. \(\rho_c\) is the medium radius of curvature of the growth step corresponding to the supersaturation \(S\) and is given by

\[
\rho_c = \frac{\sigma V_m}{kTS} \tag{57}
\]

Under the assumption that the step layer velocity is approximately equal to the growth rate and the reduction in growth rate is related to the adsorption of an impurity onto the surface it is possible to relate the degree of adsorption to the growth rate (Davey and Mullin 1974, Davey 1976). In analogy to the Langmuir isotherm, the growth rate reduction ratio can be described in terms of an adsorption isotherm (Davey and Mullin 1974, Davey 1976, Reddy 1978).

\[
\frac{V}{V_0} \approx \frac{G}{G_0} = 1 - \frac{Kc}{1 + Kc} \tag{58}
\]
As the concentration of an adsorbed impurity increases the growth rate approaches a value of zero. According to Kubota and Mullin (Kubota and Mullin 1995) one can generally not expect such behavior for all types of impurities. An effectiveness factor $\alpha$ can be introduced to compensate for non-zero growth for high concentration of impurity.

$$\frac{V}{V_0} = \frac{G}{G_0} = 1 - \alpha \frac{Kc}{1 + Kc}$$

(59)

This approach has also been used by other authors to describe the influence of an impurity on the crystal growth rate (see. e.g. Rauls et al. 2000). For $\alpha = 1$ the relative growth rate approaches zero for increasing coverage. This is shown in Figure 1.19 where a plot of relative growth rate versus dimensionless impurity concentration $Kc$ is performed.

![Figure 1.19. Relative growth rate versus dimensionless impurity.](image)

Relative growth rate versus dimensionless impurity concentration for various impurity effectiveness factors ($\alpha = 0, 0.5, 1$ and $1.5$). Source: Kubota and Mullin 1995.

According to Kubota et al. (Kubota et al. 1997) the impurity effectiveness factor is given by:
\[ \alpha = \frac{\sigma' a}{kTSL} \]  

(60)

\( \sigma' \) is the edge free energy, \( a \) the area of a growth unit, \( k \) the Boltzmann constant, the temperature, \( S \) the supersaturation and \( L \) the average spacing between active sites.

1.5 Review of Calcium Carbonate

Calcium carbonate is a white or transparent salt with a very low solubility in water. It reacts with acids forming the corresponding calcium salt and carbon dioxide. At around 900°C solid calcium carbonate dissociates into calcium oxide and carbon dioxide. In aqueous solution solid calcium carbonate is formed by a simple precipitation reaction (Schröter et al. 1990):

\[ \text{Ca}^{2+} + \text{CO}_3^{2-} \leftrightarrow \text{CaCO}_3(s) \]

Carbonic acid is not a strong acid and is under neutral pH conditions not in the form of carbonate. The ratio carbonic acid to bicarbonate ions and ration bicarbonate ions to carbonate ions is determined by the pH of the solution. Generally, at low pH only carbonic acids is present and at high pH only carbonate ions. However, the equilibrium carbonic acid/CO\(_2\) favors the latter one. As a result pH drops in a carbonate rich solution induces gassing out of carbonic acid (Schröter et al. 1990, Stumm and Morgan 1996).

Calcium carbonate precipitation preferably takes place at higher pH. During calcium carbonate precipitation, carbonate ions are withdrawn from the solution. The ratio hydrogen carbonate and carbonate ions are determined by the pH value of the solution and therefore hydrogen carbonate ions dissociate to yield carbonate ions.
Thus, the precipitation reaction lowers the pH of the solution and the dissolution of calcium carbonate increases pH.

1.5.1 Polymorphism

Calcium carbonate has elicited much interest due to its industrial importance, diversity as a biomineral, and crystalline complexity. Calcium carbonate has six polymorphic forms: three anhydrous: calcite, aragonite, and vaterite; two hydrated species: monohydrocalcite (CaCO$_3$.H$_2$O) and ikaite (CaCO$_3$.6H$_2$O); (Gaines et al. 1997) and an amorphous form. Amorphous calcium carbonate (ACC) is one of six known forms of calcium carbonate, excluding high-temperature and pressure forms. The other five crystalline, and some contain water molecules as part of their lattice structure. (Lippmann et al. 1973) Calcite and aragonite are by far the most widely produced forms of calcium carbonate in biology. (Lowenstam et al. 1989) Vaterite and monohydocalcite, although less stable, are formed by a limited number of organisms. Calcium carbonate hexahydrate, also known by its geological name ikaite, along with the other two high-temperature forms, is not as yet known to be formed biologically. ACC is the only form of calcium carbonate that is isotropic in polarized light and does not diffract X-rays; hence it is described as being amorphous.

1.5.2 Anhydrous polymorphs

Calcite is considered to be thermodynamically stable. At 25°C the solubility product of aragonite is $4.3651 \times 10^{-9}$ mol l$^{-1}$ and that of calcite $3.3497 \times 10^{-9}$ mol l$^{-1}$. The solubility product decreases with temperature (Söhnel and Garside 1992, Stumm and Morgan 1996).
Calcite has rhombohedral crystal structure with a $R\bar{3}c$ space group\(^7\). The unit cell dimensions are $a = b = 4.99$ Å, $c = 17.016$ Å, $a = b = 90^\circ$ and $g = 120^\circ$. Experimentally the \{1 0 4\} is found to be predominant one (Smyth and McCormick 1995, Stöckelmann and Hentschke 1999).

Aragonite has an orthorhombic crystal structure with a Pmcn space group. The unit cell dimensions are $a = 4.9598$ Å, $b = 7.9641$ Å, $c = 5.7379$ Å and $a = b = g = 90^\circ$. Experimentally the \{0 1 1\}, \{0 1 0\} and \{1 1 0\} are found to be the predominant faces. The \{0 1 0\} and \{1 1 0\} surfaces are dominated by layers of calcium and carbonate (Aquilano et al. 1997).

Vaterite, being considerably more unstable than both calcite and aragonite, different crystal structures have been suggested. All structures are hexagonal, yet the space group and the carbonate site symmetry differ. According to Kamhi (Kamhi 1963) vaterite is hexagonal with space group P6\(_3\). Meyer also suggests a space group P6\(_3\), but with carbonate anions in different positions. Lippmann proposes a P6\(_3\)22 space group. Generally vaterite exhibits a great deal of disorder in its crystal lattice compared to calcite and aragonite (Gabrielli et al. 2000).

1.5.3 Amorphous and Hydrated polymorphs

Calcium carbonate exhibits other solid forms besides its three true polymorphs. Clarkson and coworkers (Clarkson et al. 1992) have, for instance, dealt with the spontaneous precipitation of calcium carbonate from highly supersaturated solutions. Their findings indicate

\(^7\) The atomistic structure of crystals is of a certain anisotropic order, i.e. it depends on the direction. To describe the structure of crystals one uses geometrically well-defined elementary cells. These can e.g. be based on a hexagonal coordinate system as in the case of calcite.
that during precipitation an amorphous form of calcium carbonate is invariably formed. They also found evidence of CaCO$_3$·6H$_2$O forming under certain conditions. This is consistent with observations made by Brecevic and Nielsen (Brecevic and Nielsen 1990) who report on an amorphous form of calcium carbonate forming during spontaneous precipitation at relatively high supersaturations. The experiments show that the initial precipitate forms rapidly. It consists of particles in the size range of 50 to 400 nm in diameter that are amorphous to X-ray and electron diffraction. The particles contain less than one molecules of water per molecule of calcium carbonate. It is suggested that part of the water is a type of zeolite or crystalline lattice water. The rest is probably water included during the rapid formation of the particles. Bounded water is confirmed by infrared spectroscopy. A suggestion is that the particles form through aggregation of primary particles that are formed through primary nucleation. The solubility products determined are 6.266 (10°C), 6.393 (25°C), 6.594 (40°C) and 6.822 (55°C). X-ray and IR analysis confirm that the precursor is transformed into vaterite. Kawano and coworkers (Kawano et al. 2002), studying the precipitation of calcium carbonate from highly supersaturated solutions, have reached a similar conclusion. They found that the formation of an amorphous phase is followed by a simultaneous nucleation of both vaterite and calcite.

Gal and coworkers (Gal et al. 1996) discuss different dissolved calcium carbonate species. They suggest the existence of various hydrated forms of calcium carbonate. Their measured solubility decreases with the number of hydration water molecules. This model explains the progressive change of precipitated forms towards calcite being the most insoluble species. The hydration reactions are
endothermic coinciding with the fact that aragonite and calcite are formed on hot walls.

Other works on this topic include Koga and coworkers (Koga et al. 1998) who have studied the formation of amorphous calcium carbonate from highly supersaturated solutions and Kralj and Brecevic (Kralj and Brecevic 1995) who investigated the dissolution kinetics and solubility of CaCO₃·H₂O.

1.5.4 Phase Transformation

The phase transformation of vaterite and aragonite into stable calcite using differential scanning calorimetry has been studied by Peric and coworkers (Peric et al. 1996). The results show that the transformation reaction of mineral aragonite to calcite is relatively slow below 648 K and rapid above 698 K. The transformation is irreversible. The process is endothermic and the enthalpy of transformation is found to be 122 J g⁻¹. The activation energy is 234.5 ± 5.6 kJ mol⁻¹. The values are reportedly differ from those obtained for pure aragonite synthesized in the laboratory (296.4 kJ mol⁻¹ and 50 Jg⁻¹). The irreversible transformation of vaterite to calcite appears upon heating above 730 K. The activation energy is 252.8 ± 48.7 kJ mol⁻¹ and the enthalpy of transformation is -21.2 Jg⁻¹.

Kralji and coworkers (Kralji et al. 1997) have studied the kinetics of transformation of vaterite to calcite in aqueous solution in the temperature range of 25 to 45°C by recording pH. The observations made led to the conclusion that the transformation is solution-mediated and that the rate is determined by the growth of calcite. Spanos et al. (Spanos and Koutsoukos 1998) have performed similar studies on the phase transformation of vaterite to calcite during
precipitation of calcium carbonate. pH and temperatures in the range of 25 to 45°C do not have any significant influence on the transformation. The rate of transformation decreases as the rate of supersaturation increases. The transformation may be explained by the dissolution of small vaterite crystals followed by the crystallization of calcite.

1.5.5 Crystal Growth and Dissolution

Liang and co-workers (Liang et al. 1996) have studied the dissolution kinetics at the calcite {1 0 4} water interface using a combined experimental and theoretical approach, including atomic force microscopy, an analytical terrace-ledge-kink model and kinetic Monte-Carlo simulations.

Reddy and Nancollas (Reddy and Nancollas 1971) have studied the kinetics of calcite growth by seeding with to calcite metastable solutions. Their results indicate that calcite growth is a surface controlled process following a second order rate equation with respect to the concentration. Christoffersen and Christoffersen (Christoffersen and Christoffersen 1990) have performed pH-stat seeded growth experiments of calcite. The experimental results indicate that calcite grows according to a spiral growth mechanism. The absolute rate constant obtained is a factor 2 smaller than the maximum rate constant that can be obtained by predicting activation energy for the process. Gutjahr and coworkers (Gutjahr et al. 1996a) have studied the growth kinetics of calcite and aragonite in the temperature range of 20 to 70°C at different calcium and carbonate concentration levels. At moderate super- and undersaturation levels growth and dissolution are determined by processes at the crystal surface and not by diffusion.
Growth and dissolution rates do not depend on the individual concentration of calcium or carbonate, but entirely on the ion product. At higher super- and undersaturation levels the formation of calcium carbonate governs the growth rate. For aragonite the interpretation of the experimental results is difficult due to the existence of different crystallographic faces. Gómez-Morales and co-workers (Gómez-Morales et al. 1998) using seeded pH-stat and free drift experiments have also studied the crystal growth of calcite at different temperatures. The results of the pH-stat experiments indicate that the growth mechanism changes with temperature.

Kazmierczak and coworkers (Kazmierczak et al. 1982) were the first to use a controlled composition method to study the kinetics of calcium carbonate crystallization. The activities of ionic species in the supersaturated solution are maintained constant by the potentiometrically controlled addition of reagents. The rate of crystallization is found to be proportional to the square of the supersaturation expressed in terms of activities of free calcium and carbonate ions. This points towards a surface controlled reaction. Parsiegla and Katz (Parsiegla and Katz 1999) have also studied the crystal growth of calcite using a constant composition method. The order of the calcite growth rate depends on the solution composition and ranged from 1.1 to 1.7. Atomic force microscopy showed that calcite predominantly grows by a screw dislocation mechanism.

Kralj and coworkers (Kralj et al. 1990, Kralj et al. 1994, Kralj et al. 1997) have in three papers dealt with growth and dissolution of vaterite in aqueous solutions. The first paper focuses on the kinetics. This was done by recording the pH during spontaneous precipitation. The results combined with numerical treatment indicate that the
crystal growth kinetics are parabolic in accordance with the theory for screw dislocation surface spiral controlled growth of electrolytes in aqueous systems. The results of their investigation of the dissolution kinetics show that these are governed by the diffusion of the hydrated constituent ions, i.e. Ca\(^{2+}\) and CO\(_{3}^{2-}\), away from the surface. This conclusion coincides with the dependence of the dissolution rate on the concentration of the constituents in the solution.

Nilsson and Sternbeck (Nilsson and Sternbeck 1999) have developed a new mechanistic model for the crystal growth kinetics of calcite. It accounts for the presence of various surface complexes. Calcite growth is assumed to occur through three reversible reactions. The results of the model indicate that CaCO\(_3\) (aq) is roughly 20 times more active than Ca\(^{2+}\) (aq) at the calcite water surface.

1.5.6 Biomimetic Methods for Synthesis of CaCO\(_3\)

Many living organisms are well able to synthesize highly optimized composite materials by means of template controlled mineralization like pearls, shells, etc. For example, amorphous calcium carbonate (ACC) deposits of *Porcellio scaber* (land-based woodlice) are built by numerous spherules and associated with an organic matrix consisting of radial and concentric elements, suggesting that the organic components function as a template for spherule formation. This process is known as *Biomineralization*. Recent efforts in the laboratory to duplicate this natural method of synthesis have come to be known as *biomimetic methods for synthesis*. An understanding of the mechanism involved in such a matrix-mediated synthesis has been recognized to be of great potential in the production of engineering materials. In the present study, *Porcellio scaber* is used as a model to
investigate the dissolution behavior of sternal ACC deposits by atomic force microscopy which provides a better way to understand biomineralization at molecular level.

Such biological systems manage to control crystal growth to high degrees of specificity by using a wide range of natural additives; from basic metal ions to complex organic polymers. Organic polymers control crystal growth, habit and morphology by controlling the crystal macrostructure through intermolecular interactions at nanoscale. Biopolymers can also adsorb onto crystal surfaces, altering the stability of that face and hence affecting the growth rate. These organic regulators are often identified as polymers that contain repeat units of distinct peptides, or amino acids, in their primary sequence. The question which remains to be answered is the nature of the crystal-organic interactions that mediate these effects. In this context, an examination of how CaCO$_3$ growth is affected by water-soluble polymers and low molecular weight additives will provide further insight and information.

Proteins and polypeptides possess carboxylic acid groups in their side chains and are known to shape biogenic CaCO$_3$. Because of this, related additives were selected which contains acidic groups available to interact with other molecules or surfaces. It is widely accepted that the acidic functional groups adsorb onto the surface of calcite. It is generally proposed that mimetic substitution by the carboxylic acid groups for the carbonate ions on the calcite surface slows the growth of the higher energy, faster growing faces, hence altering the crystal habit and morphology. Organisms generally utilize complex mixture of crystal modifiers rather than a simple additive to generate the variety of biomaterials observed. To begin to address this,
I have investigated the effect of water-soluble polymers with amide, carboxylic and sulphonic acid functionalities, on the crystallization behavior of CaCO₃. The polymers used were acrylamide based on acrylamido methyl propane sulphonic acid with varying monomer compositions and monomeric units. For the desired purpose of ion binding, several low molecular weight additives such as simple amino acids, peptides, proteins, inorganic salts were also examined in combination with polymers.

1.5.7 Easy Approach for the Synthesis of Calcium Carbonate (CaCO₃)

Synthesis of calcium carbonate was followed by two basic synthetic routes: double decomposition reaction (a simple route, but it is difficult to control the shape and modification of CaCO₃) and miniemulsion method (CaCO₃ synthesis was performed in 10¹⁸ - 10²⁰ nanocompartments which are separated from each other by a continuous phase). ACC is a fascinating form of CaCO₃ that may well be of much interest to materials science and biomineralization. ACC in its pure form is highly unstable, yet some organisms produce stable ACC, and cases are known in which ACC function as a transient precursor of more stable crystalline CaCO₃.

By using miniemulsion technique, I have synthesized an ACC nanoparticle which was stable in solution and also in dried state for a several days.
Chapter 2

CaCO₃ Crystal Modifications
Using Water-soluble Polymers
Based on AMPS Monomer

The effect of water-soluble polymers on the crystallization of calcium carbonate has been reported. The polymers used were acrylamide based on AMPS monomer with varying monomer compositions and monomeric units. For the desired purpose of ion binding, several low molecular weight additives such as simple amino acids, peptides, proteins, inorganic salts were also examined in combination with polymers. Crystallization began by mixing together, with or without a polymer or other additive, calcium chloride and sodium/potassium carbonate. The first crystals to form in most cases were vaterite, possibly with a very small amount of calcite. Subsequent behavior depended on the polymer or other additive present. With most additives, the vaterite transformed, within ca. 1 h, to calcite probably by dissolution and recrystallization.

2.1 Introduction

2.1.1 Direction of research

Water soluble polymers have been attracting a great deal of attention due to their utility in several industrial applications. Polyacrylamide (Lezovic et al. 1998, Erim et al. 1992, Pabon et al. 1999, Barajas et al. 1997) and acrylamide co and terpolymers (Rivas et al. 1999, Ye et al. 1999)
occupy a unique position in water soluble polymers due to their several advantageous properties and are used in mineralization process for crystal habit modifications (Pai et al. 2004). Low cost of acrylamide polymers makes mineralization process economically reasonable. Acrylamido methyl propane sulphonic acid (AMPS) polymers and co and terpolymers figure prominently under water soluble polymers due to their versatile properties (Rivas et al. 1999, Ye et al. 1999). AMPS are a highly reactive sulphonic acid vinyl monomer from which a wide variety of polymers can be prepared. These polymers offer excellent hydrolytic stability and show high divalent cation tolerance. A large number of patent literatures (U.S. Pat. No. 5,326,854; U.S. Pat. No. 2003/0031643A1; U.S. Pat. No. 4,544,722. 1885; U.S. Pat. No. 5,366,729. 1994; U.S. Pat. No. 5,260,391. 1993) exist on the synthesis and applications of AMPS based polymers.

The main objective of the present study is to investigate the role of synthetic water soluble polymers in controlling calcium carbonate deposition; I have studied the effect that simple additives exert over calcium carbonate growth from aqueous solution (i.e., polymorphic forms and resultant crystal morphologies). A number of additives such as simple amino acids, peptides, inorganic salts, and bioorganic macromolecules (Pai et al. 2004) have been used as crystal modifiers in addition to synthetic water soluble polymer. Organisms generally utilize complex mixture of crystal modifiers, rather than a simple additive, to generate the variety of biomaterials observed. To begin to address this, I have investigated the possible synergism that arises when both the synthetic water soluble polymer and additive are present (Pai et al. 2004). The overall work and synthetic strategy includes the preparation of various polymorphic forms and resultant
CaCO$_3$ crystal morphologies by double decomposition precipitation method (Pai et al. 2004).

2.1.2 Performance properties of water soluble polymers

The performance properties of acrylamide based water soluble polymers are dependent on molecular weight, functional groups (Pai et al. 2004) and the experimental conditions such as the type of the salts used (Dragan et al. 2003), their concentration (Melekaslan et al. 2000), pH, temperature, rate of mixing the solutions and the intensity of agitation of the reaction mixture. Hence suitable compositions of some monomers such as AMPS (acrylamido methyl propane sulphonic acid), NVP (n-vinyl pyrrolidone), VA (vinyl alcohol) and SA (sodium acrylate) monomer will be optimized for acceptable performance. Acrylamido methyl propane sulphonic acid (AMPS) monomer can be homopolymerized and copolymerized with other vinyl monomers (U.S. Pat. No. 5,080,809. 1985; U.S. Pat. No. 4,951,921. 1984; U.S. Pat. No. 4,644,020. 1983; U.S. Pat. No. 3,146,193. 1964). AMPS and their Na salts show good reactivity in free radical homo and copolymerization. They display strong polyelectrolyte behavior and show large tolerance to divalent cations compared to carboxylated polymers.

Aqueous solutions of polymers are quite stable and show enhanced hydrolytic stability due to shielding of sulphonic acid group by the monomers unique dimethyl structure. The polymers have outstanding thermal properties. The main aim of the present study is to demonstrate the feasibility of well-designed co and terpolymers (Pai et al. 2004) for CaCO$_3$ crystal modifications. The ultimate behavior of these water soluble polymers in aqueous solution is difficult to predict because, they form complex with salt solution (McCormick et al. 1984).
Factors such as temperature, interaction with ionic species and the relative size of the chain molecules (Renken et al. 1999) are all important parameters and need to be considered.

2.1.3 Synthetic strategy

The terpolymers of AM 80: NaAMPS 10: NVP 10 mol% and AM 70: NaAMPS 20: NVP 10 mol% were prepared using 4, 4' azobis (4-cyanovaleric acid) (Figure 2.1) initiator with 40% monomer concentrations. The relative viscosity was 3.05 and 1.88 and the conversions were 95 and 90% respectively. These samples form good hard gel and take a long time (more than 72 hrs) for dissolution and low amount of insoluble part was present. Terpolymers of equimolar ratio of AM 33.33: NaAMPS 33.33: NVP 33.33 mol% and AM 80: NaAMPS 10: SA 10 mol% (Figure 2.1) and AM 80: NaAMPS 10: VA 10 mol% (Figure 2.1) were prepared using 4-cyanovaleric acid initiator with 40% monomer concentration.

The relative viscosity was 2.06, 2.2 and 2.1 and the conversions were 96, 80 and 75% respectively. It was observed that the free radical solution polymerization of AMPS or AM 85: AMPS 15 with 40% monomer concentration resulted in thick polymer mass and the molecular weight obtained are less than 5 million. Experiments carried out to increase the molecular weight by inverse emulsion polymerization were not successful due to poor stability of the emulsions formed. This is mainly because of highly acid nature of AMPS monomer. Detailed literature search using SciFinder was carried out and new surfactant and initiators were identified.
Copolymers of AM 85 and AMPS 15 (Figure 2.1) were prepared by both free radical and inverse emulsion polymerization techniques using a special initiator V-50 and a combination of redox initiator with 40% monomer concentration. In inverse emulsion polymerization both oil and water soluble initiators and suitable surfactant combinations including polymeric type were used to achieve high molecular weights. Similar results were obtained in the preparation of AM 85: NaAMPS 15
mole% (Figure 2.1) copolymer. Copolymers of NaAMPS 80 and 95 with NVP 20 and 5 mole% (Figure 2.1) were prepared by free radical solution polymerization using K$_2$S$_2$O$_8$ as thermal initiator. The AMPS content in the samples was determined by $^{13}$C NMR measurements. The relative viscosity obtained for these samples were in the required range. Samples with AMPS content 80-95 mole% resulted in good hard gel. These samples are stable at 130°C for over 6 months. (Courtesy: Dr. R. A. Kulkarni, Department of Polymer Chemistry, NCL, Pune, India: for giving me the guidance to synthesize acrylamide based water-soluble polymers and permission to use these polymers for my Ph.D. work.)

2.2 Experimental Section

2.2.1 Materials

All chemicals were of analytical grade and used without further purification.

2.2.2 Methods

(a) Crystallization of calcium carbonates.

The precipitation of CaCO$_3$ was carried out in a round bottom flask (RBF) at -20°C in order to stabilise less stable polymorph, by reducing the rate of transformation to calcite (Figure 2.2). About 45 mg of polymer was transferred into a 50 ml RBF and 3 ml of 2 M CaCl$_2$ was added. The polymer was allowed to swell overnight. The solution was homogenized the next day using a magnetic stirrer and the pH was adjusted to 8.5 using 0.1 N NaOH. This solution was kept under N$_2$ to
prevent the dissolution of CO$_2$ from the air. 3 ml of 2 M K$_2$CO$_3$ / Na$_2$CO$_3$ (pH 11) was added dropwise under continuous stirring.

**Figure 2.2. Double decomposition precipitation method.** Experimental set-up for the preparation of various forms of CaCO$_3$ crystal morphologies by double decomposition precipitation method.

After complete addition of K$_2$CO$_3$ / Na$_2$CO$_3$ the polymer solution was kept for one week at -20°C to allow crystal growth. The polymer solution containing the CaCO$_3$ precipitate was filtered through a 22µm pore size filter media and washed three times with Millipore water. Finally, the product was air dried at room temperature. To study the role of additives (Figure 2.3) in the CaCO$_3$ crystallization, 20 mg of poly-Asp, L-glutamine and N-acetyl-L-glutamic acid (monomers), glutathione (tri-peptide) were dissolved in 3 ml 2 M CaCl$_2$ solution. I also tested the effect of magnesium ions on
CaCO$_3$ precipitation by adding 100 mM magnesium chloride to polymer solution. In control experiment the additives were omitted.

![Chemical structures of L. Glutamine, N-Acetyl-L-Glutamic acid, Glutathione, and Poly(Aspartic acid)](image_url)

**Figure 2.3. Chemical structure of Additives.** Chemical structure of additives used in the precipitation of CaCO$_3$.

**(b) Field Emission Scanning Electron Microscopy (FESEM)**

SEM studies on the CaCO$_3$ crystals were carried out using a Hitachi S-5200 field-emission scanning electron microscope at acceleration voltage of 15 or 20 kV after rotary coating with 3nm of platinum at an angle of 45$^\circ$ in a Balzers BAF 300. Since the experimental solutions contain KCl, we performed energy dispersive spectroscopy to analyse the chemical composition of the crystals. The results verified the precipitate is CaCO$_3$ with only little / no K and Cl present (Courtesy: PD Dr. Ziegler for SEM analysis).
(c) Calcium ion measurements

To estimate the polymer / CaCO$_3$ ratio, we dissolved an aliquot of sample in 0.1 M KCl adjusted to a PH of 6.0 and measured the Ca$^{2+}$ concentration using Ca$^{2+}$ selective mini electrodes (ETH 1001). We use the calibration solutions according to Tsien, R.Y. and Rink T.J. Since ionic strength in our sample and test solutions were equal Ca$^{2+}$ concentration rather than Ca$^{2+}$ activity was determined. The results show that in all samples the amount of polymer was less than 20% (Courtesy: PD Dr. Ziegler for analysis).

(d) X-ray Diffraction Studies

Powder X-ray diffraction studies on the crystal powder were carried out using a Siemens D500 X-ray diffractometer with Cu K$_\alpha$ radiation at 40 kV and 30 mA (Courtesy: Dr. Bernd Heise and Gerda Dörfler for XRD analysis).

(e) Infrared spectroscopy (FTIR)

Spectra were recorded on a Bruker FT/IR-IFS 113V spectrometer. Samples were examined in potassium bromide discs (Courtesy: Elvira Kaltenecker for FTIR analysis).

(f) Raman spectroscopy

Raman spectra were recorded using a CRM 200 confocal Raman spectrometer, WiTec, Ulm, Germany. The exciting source was a SGL-2200 laser operating at 532 nm with a power of about 200 mW. The scattered light was collected at an angle of 180° (back-scattering). Typical spectral resolution was 3cm$^{-1}$. To avoid the transformation of less stable polymorphs into calcite the laser beam is scanned in a field
of 200 x 200 µm². The total exposure time was 20 sec. Original spectra
are normalized to the calcite peak at 280 cm⁻¹ (kind thanks Alexander
Otten, Abteilung Angewandte Physik for helping me for Raman
analysis and also thank to Dr. Hild for helping me for normalizing the
spectra).

2.3 Results and Discussion

2.3.1 Crystals precipitated in the polymer-free control
experiments

Figure 2.4. SEM micrographs of CaCO₃ crystals. CaCO₃ mineralization in
the absence of additives which results in rhombohedral calcite crystals.

CaCO₃ precipitation in the absence of additives or polymer
resulted in the calcite rhombohedral morphologies (Figure 2.4.). The
introduction of additives or polymer or in combination into the
solution resulted in the precipitation of other modifications and the
morphology of the calcite crystals also changed from regular
rhombohedra to elongated crystal aggregates of various type which is
discussed one by one in the following sections.
2.3.2 Crystals precipitated in the presence of terpolymer with varying mole percentage of monomeric units

Figure 2.5 shows the typical electron microscopy (EM) pictures of CaCO₃ particles obtained in the presence of the synthetic water-soluble poly (acrylamide-co-acrylamido-2-methyl-1-propane sodium sulfonate-co-n-vinyl pyrrolidone) [poly (AM-NaAMPS-NVP)] with monomer compositions of AM 33.33 : NaAMPS 33.33 : NVP 33.33, AM 80 : NaAMPS 10 : NVP 10, and AM 70 : NaAMPS 20 : NVP 10 mole% respectively under the standard synthesis condition with polymer concentration of 0.2 gL⁻¹, calcium chloride & potassium carbonate concentration of 2 M, and a starting pH of 7.5.

Similar to the case of the control experiments in which no polymeric additives were present, regular rhombohedral crystals characteristic of calcite were produced in the absence of polymer and additives, as shown in Figure 2.4; this suggests the necessity of polymer and additives for controlling the crystal growth. Both monomeric units and composition retarded the nucleation and growth rate of CaCO₃ (the effect increasing with the percentage composition of monomeric unit present) with the equimolar ratio (AM 33.33: NaAMPS 33.33: NVP 33.33) having a more pronounced effect than other two (AM 80: NaAMPS 10: NVP 10, and AM 70: NaAMPS 20: NVP 10 mole%). At highest mole % of NaAMPS content in the terpolymer composition (AM 70: NaAMPS 20: NVP 10 mole %) leads to staked rhombohedral crystals aggregates in the size range between 20-30 µm (Figure 2.5Ca & Cb). The end faces of the crystal were roughened that were often poorly-defined and exhibited large indentations.
Figure 2.5. SEM micrographs of CaCO₃ crystals. CaCO₃ mineralization in the presence of (Aa & Ab) Poly (AM 33.33: NaAMPS 33.33: NVP 33.33) mole%, (Ba & Bb) Poly (AM 80: NaAMPS 10: NVP 10) mole%, and (Ca & Cb) Poly (AM 70: NaAMPS 20: NVP 10) mole% respectively. Higher magnification shows etch pits and indentations are considerably decreased and disappeared when equimolar ratio of monomeric units in the terpolymer is used for mineralization.
Here the tendency to form spherical crystal aggregates was less pronounced compared to other two (Figure 2.6cc).

![Image](204x546.png)

**Figure 2.6. SEM micrographs of CaCO₃ crystals.** CaCO₃ mineralization in the presence of (aa) Poly (AM 33.33: NaAMPS 33.33: NVP 33.33) mole %, (bb) Poly (AM 80: NaAMPS 10: NVP 10) mole %, and (cc) Poly (AM 70: NaAMPS 20: NVP 10) mole % respectively.

FTIR analysis of the obtained crystals aggregates reveals only the presence of calcite modification (Figure 2.7c). With decreasing mole % of monomeric content in the terpolymer composition (AM 80: NaAMPS 10: NVP 10 and AM 33.33: NaAMPS 33.33: NVP 33.33) the crystals size became smaller as well as the tendency to form spherical crystal aggregates increases considerably (Figure 2.5Ba & Bb and Aa & Ab). The rhombohedral crystal aggregates obtained from AM 80: NaAMPS 10: NVP 10 mole % was spherical in morphology and in the
size range between 20-25 µm (Figure 2.6bb). Few staked rhombohedral crystals aggregates were obtained as in the case before (AM 70: NaAMPS 20: NVP 10 mole %). FTIR analysis of the samples AM 80: NaAMPS 10: NVP 10 mole % shows only the presence of calcite (Figure 2.7b).

**Table 1. FTIR peaks from experimental data cm⁻¹**

<table>
<thead>
<tr>
<th>Modifications</th>
<th>Reference peak cm⁻¹</th>
<th>FTIR peaks from experimental data cm⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcite #</td>
<td>2972</td>
<td>2872</td>
</tr>
<tr>
<td></td>
<td>2515</td>
<td>2515</td>
</tr>
<tr>
<td></td>
<td>1918</td>
<td>2516</td>
</tr>
<tr>
<td></td>
<td>719</td>
<td>712</td>
</tr>
<tr>
<td></td>
<td>877/713</td>
<td>877/713</td>
</tr>
<tr>
<td>Vaterite $b$</td>
<td>2967</td>
<td>2967</td>
</tr>
<tr>
<td></td>
<td>2256</td>
<td>2256</td>
</tr>
<tr>
<td></td>
<td>1490,1432</td>
<td>1490,1432</td>
</tr>
<tr>
<td></td>
<td>1089,877/745</td>
<td>1089,877/745</td>
</tr>
</tbody>
</table>

*Figure 2.7. Summary of the experimental results. Left) FTIR analysis shows summary of the experimental results of the nucleation of CaCO₃ modifications and right) IR spectra for the corresponding analysis: in the presence of (a) Poly (AM 70: NaAMPS 20: NVP 10), (b) Poly (AM 80: NaAMPS 10: NVP 10), (c) Poly (AM 33.33: NaAMPS 33.33: NVP 33.33) mole% respectively.

Equimolar ratio (AM 33.33: NaAMPS 33.33: NVP 33.33) exerted a strong morphological influence on growing CaCO₃ crystals. A distinct morphological change was evident in these samples. Crystal aggregates were spherical in morphology in the size range between 2-10 µm (Figure 2.5Aa & Ab). Here the crystals are much smaller compared to other two cases. Closer examination of these spherical surfaces revealed it is smooth and composed of several tiny uniform

---

8 #Weir, *et al.* (1961); #White, *et al.* (1974)
spheres in the size range between 150-200 nm (Figure 2.6aa). As in the other two cases very few stacked rhombohedral crystal aggregates were found, but the faces were smooth and exhibited no indentations. FTIR analysis (Figure 2.7a) of these samples shows both the presence of calcite and vaterite but latter is the predominant phase.

2.3.3 Crystals precipitated in the presence of terpolymer with different carbonate salts

The influence of two types of carbonate salts (Na$_2$CO$_3$ and K$_2$CO$_3$) on the growth of CaCO$_3$ in the presence of same terpolymer (AM 80: NaAMPS 10: NVP 10 mole %) under similar reaction conditions was more striking. Both salts (Na$_2$CO$_3$ and K$_2$CO$_3$) retarded the nucleation and growth rate of CaCO$_3$ and exerted a strong morphological influence on growing calcite crystal aggregates. A distinct morphological change was evident in both samples containing Na$_2$CO$_3$ and K$_2$CO$_3$ respectively. Reaction mixtures comprising aqueous calcium chloride and sodium carbonate was prepared at a [Ca$^{2+}$]: [CO$_3^{2-}$] molar ratio of 1:1 and pH value of 8.2 and stirred for 2 min, the crystallization of CaCO$_3$ resulted in stacked crystal aggregates with a mean size of 10 µm (Figure 2.8A & a). The obtained particles are rather uniform in size and shape, with (104) rhombohedral faces and this was confirmed by XRD (Pai et al. 2004). Whereas the reaction carried out using potassium carbonate under the same reaction conditions, resulted in rhombohedral crystal aggregates of spherical morphology and in the size range between 20-25 µm (Figure 2.8B&b). There are few staked rhombohedral crystal aggregates and end faces of the crystal were roughened that were often poorly-defined and
exhibited large indentations. Here the crystal size were much larger compared to the other. Selected area X-ray diffraction, as well as FTIR indicated that the CaCO$_3$ were calcite modification.

**Figure 2.8. SEM micrographs of CaCO$_3$ crystals.** CaCO$_3$ mineralization in the presence of (A & a) Poly (AM 80: NaAMPS 10: NVP 10) mole% (Na$_2$CO$_3$ + CaCl$_2$), (B & b) Poly (AM 80: NaAMPS 10: NVP 10) mole% (K$_2$CO$_3$ + CaCl$_2$) respectively.

2.3.4 Crystals precipitated in the presence of terpolymer with the replacement from SA to NVP unit

When the synthetic poly (AM-NaAMPS-SA) and poly (AM-NaAMPS-NVP) with monomer compositions of 80:10:10 mole %, was used as an additive, the crystallization of CaCO$_3$ resulted in crystal aggregates, and a distinct morphological change was evident in both cases. Crystallization in the presence of poly (AM-NaAMPS-SA) results
in the formation of aggregates of spherical morphology with diameters between 5 and 10 µm (Figure 2.9A).

Figure 2.9. SEM micrographs of CaCO₃ crystals. CaCO₃ mineralization in the presence of (A) Poly (AM 80: NaAMPS 10: SA 10) mole%, (B) Poly (AM 80: NaAMPS 10: NVP 10) mole% respectively.

The spheres consist of rhombohedral sub crystals, with sizes in the range of 500 nm. FTIR, XRD and Raman analysis shows the presence of calcite (Figure 2.25C-2, 2.35e and 2.36a respectively). Whereas, crystallization in presence of poly (AM-NaAMPS-NVP) results in the formation of stacked crystal aggregates with a mean size of 10 µm (Figure 2.9B). The obtained particles are rather uniform in size and shape, with (104) rhombohedral faces and this was confirmed by XRD.

2.3.5 Crystals precipitated in the presence of copolymer with the replacement from AMPS to NaAMPS unit

When the copolymer of poly (AM-AMPS) with monomer composition 85:15 mole % was used as an additive, the crystallization of CaCO₃ resulted in crystals that exhibited different morphologies, that is rhombohedral crystal spherical aggregates with rough surface (Figure 2.10Aa), spheres with smooth surface (Figure 2.10Ab), irregularly intergrown lobes and spheres (Figure 2.10Ac), and aggregates of complex forms, including stepped rhombs, with terraces,
likely linked by \{00.1\} faces (Figure 2.10Ad) is found. FTIR analysis (Figure 2.11) shows both the calcite and aragonite are present.

Figure 2.10. SEM micrographs of CaCO$_3$ crystals. CaCO$_3$ mineralization in the presence of (Aa-Ad) Poly (AM 85: AMPS 15) and (Ba-Bb) Poly (AM 85: NaAMPS 15) mole\% respectively.
Whereas AMPS unit is replaced by NaAMPS in the copolymer composition, the crystallization of CaCO₃ resulted in irregular rhombohedral crystals aggregates and end faces of the crystal were roughened that were often poorly-defined and exhibited large notches. Several hollow spheres (2-6 µm), in addition to the irregular rhombohedral species were obtained (Figure 2.10Ba & Bb).

<table>
<thead>
<tr>
<th>Modifications</th>
<th>Reference peak cm⁻¹</th>
<th>FTIR peaks from experimental data cm⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Poly (AM-AMPS)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Poly (AM-AMPS)</td>
</tr>
<tr>
<td>Calcite</td>
<td>2872, 2814, 1012</td>
<td>2872, 2814, 1012</td>
</tr>
<tr>
<td></td>
<td>877/718</td>
<td>877/718, 879/714</td>
</tr>
<tr>
<td>Vaterite</td>
<td>1409/1089, 877/745</td>
<td>1409/1089, 879/745</td>
</tr>
<tr>
<td>Aragonite</td>
<td>2854, 2546, 1083</td>
<td>2854, 2546</td>
</tr>
<tr>
<td></td>
<td>857/713</td>
<td>857/713</td>
</tr>
</tbody>
</table>

**Figure 2.11. Summary of the experimental results.** Left) FTIR analysis shows summary of the experimental results of the nucleation of CaCO₃ modifications and right) IR spectra for the corresponding analysis: in the presence of Poly (AM 85: AMPS 15) and Poly (AM 85: NaAMPS 15) mole% respectively.

The FTIR analysis of this sample is shown in Figure 2.11, which shows weak and broad peak from vaterite, a metastable polymorph of CaCO₃, in addition to the sharp peaks from calcite.

---

9 #Weir, et al. (1961); † #White, et al. (1974)
2.3.6 Crystals precipitated in the presence of copolymer with varying mole percentage of monomeric units

Figure 2.12 shows the typical electron microscopy (EM) pictures of CaCO₃ particles obtained in the presence of the synthetic water-soluble poly (acrylamido-2-methyl-1-propane sodium sulfonate-co-n-vinyl pyrrolidone) [poly (NaAMPS-NVP)] with monomer compositions of NaAMPS 80 : NVP 20 and NaAMPS 95 : NVP 5 mole % respectively.

![SEM micrographs of CaCO₃ crystals.](image)

Copolymer with NaAMPS 80: NVP 20 mole % exerted an influence on crystal morphology producing crystals that were...
CaCO₃ Crystal Modifications Using Water-soluble Polymers Based on AMPS Monomer

... elongated rock-like and one end of the crystal face was smooth and other faces were roughened that were poorly-defined (Figure 2.12Aa &Ab). Closer examination of these surfaces revealed stepped rhombs, with terraces containing faces. Whereas the CaCO₃ particles obtained in the presence NaAMPS 95: NVP 5 mole % resulted in irregular rhombohedral spherical aggregates and faces of the crystal were stepped rhombs and exhibited large net-like faces (Figure 2.12Ba &Bb). The FTIR analysis is shown in Figure 2.13, which shows peak from vaterite & calcite in the sample NaAMPS 80: NVP 20 and only calcite in NaAMPS 95: NVP 5 respectively.

![Figure 2.13. Summary of the experimental results. Left) FTIR analysis shows summary of the experimental results of the nucleation of CaCO₃ modifications and right) IR spectra for the corresponding analysis: in the presence of poly (NaAMPS 80: NVP 20) and poly (NaAMPS 95: NVP 5) mole% respectively.](image)

2.3.7 Water-soluble terpolymer direct the hollow triangular cones of packed calcite needles

Here, I report hollow triangular cones of packed needles, which have never been seen in natural biominerals, were prepared by a

---

10 ‡Weir, et al. (1961); †White, et al. (1974)
simple double decomposition precipitation method in the presence of poly (acrylamide-co-acrylamido-2-methyl-1-propane sodium sulfonate-co-vinyl alcohol) [poly (AM-NaAMPS-VA)] (relative viscosity ~ 2) with monomer compositions of 80:10:10 mole % as a crystal modifier. In a typical synthesis, a solution of CaCl₂ (2M, 2ml) was added into an aqueous solution of Poly (AM-NaAMPS-VA) (100 mL, 2.0 gL⁻¹) and the pH of the solution was adjusted to 7.5 by using HCl or NaOH. Then a solution of K₂CO₃ (2M, 2ml) was added dropwise into the pH-adjusted solution under vigorous stirring for 1 min, and then the solution was kept under static condition in refrigerator at -20°C for one week.

![Figure 2.14. SEM micrographs of CaCO₃ crystals.](image)

CaCO₃ mineralization in the presence of poly (AM-NaAMPS-VA), (B-D) higher-magnification showing detailed texture.
Figure 2.15. SEM micrographs of CaCO$_3$ crystals. CaCO$_3$ mineralization in the presence of poly (AM-NaAMPS-VA), (A-D) higher-magnification SEM images showing detailed texture and porosities.

Figure 2.14 – 2.16 shows typical scanning electron microscope images of the as-prepared samples obtained in the presence of Poly (AM-NaAMPS-VA) (100 mL, 2.0 gL$^{-1}$) after aging at room temperature for several months. The crystallization of CaCO$_3$ resulted in crystals that exhibited different morphologies, which are spheres in the size range between 250-700 nm (Figure 2.16A), hollow spheres in the size range between 120-240 nm (Figure 2.16B), unique spherical granular (size ~95) aggregates with stepped rhombs (Figure 2.16C), rock-like crystals in the size range between 15-20 µm (Figure 2.16D), and several spherical superstructures (Figure 2.14 and 2.15). The spherical superstructures are about 40-45 µm in size and their surface is composed of triangular-shaped cones of packed calcite needles that are
in the size range between 400 nm - 3 µm (triangle size) (Figure 2.14 and 2.15) and the length of the cone (bottom of the triangle) is 700 nm - 1.5 µm (Figure 2.14D), and aligned on the surface of the spheres.

![SEM micrographs of CaCO₃ crystals.](image)

**Figure 2.16.** SEM micrographs of CaCO₃ crystals. CaCO₃ mineralization in the presence of poly (AM-NaAMPS-VA), (A-D) higher-magnification SEM images showing detailed texture and porosities.

This implies a multistep growth mechanism where at first small spheres (or nuclei) are formed which is then followed by overgrowth of the spheres by self-organization of smaller particles on their surface (Yu et al. 2004). The high-magnification SEM images show that the microsphere appears to be higher-order porous triangular-shaped cones of packed calcite needles (Figure 2.15A-D). An FTIR analysis of the obtained samples indicates that the mixtures of calcite, aragonite and CaCO₃.6H₂O (ikaita) modifications are present (Figure 2.17).
CaCO$_3$ Crystal Modifications Using Water-soluble Polymers Based on AMPS Monomer

Table 1

<table>
<thead>
<tr>
<th>Modifications</th>
<th>Reference peak cm$^{-1}$</th>
<th>FTIR peaks from experimental data cm$^{-1}$ Poly (AM-NaAMPS-VA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcite$^*$</td>
<td>2755 2799 1425</td>
<td>2757 1425</td>
</tr>
<tr>
<td>Aragonite$^*$</td>
<td>1477 1294 1109 1089 587</td>
<td>1477 1294 1109 1089 587</td>
</tr>
<tr>
<td>CaCO$_3$:0.4H$_2$O$^†$</td>
<td>2755 2799 1425</td>
<td>2757 1425</td>
</tr>
</tbody>
</table>

Figure 2.17. Summary of the experimental results. Left) FTIR analysis shows summary of the experimental results of the nucleation of CaCO$_3$ modifications and right) IR spectra$^{11}$ for the corresponding analysis: in the presence of poly (AM- NaAMPS-VA).

According to Yu and coworkers (Yu et al. 2004) the mechanism can take place in two-step process. In the first step, the chains of Poly (AM-NaAMPS-VA) accumulate electrostatically a large amount of Ca$^{2+}$ and carbonate ions. Then nucleation occurs at an outburst speed forming a large number of small calcite and vaterite particles. These particles aggregate by the covalent interactions between Ca$^{2+}$ ions on the surface of CaCO$_3$ and functional groups (amide, hydroxyl and sulphonic acid) on the polymer chains (Yu et al. 2002 and Park et al. 2001). When the growth of CaCO$_3$ particles reaches a critical size, the inorganic/organic composite particles separate from solution due to phase separation and generally adopt a spherical morphology because this gives the minimum total surface energy for a given volume (Yu et

---

$^{11}$Kennedy, et al. (1987); $^†$White, et al. (1974)
al. 2004). In the second step, the functional groups (amide, hydroxyl and sulphonic acid) are adsorbed and self-assembled on the surface of the as-formed composite microspheres. Mann, et al and Heywood, et al (Mann et al. 1998 and Heywood et al. 1994) proposed that this kind of environment may be more suitable for the growth of rhombohedral calcite crystals, which are often nucleated on a small triangular (001) face at a rigid substrate surface. Such secondary nucleation would results in the formation of many small calcite crystals with a trigonal pyramidal morphology (Williamson et al. 1968). The deposition of the precise array of calcite crystals on the surface of composite micspheres is ascribed to the controlled nucleation at the interface between the crystals and the adsorbed functional groups (amide, hydroxyl and sulphonic acid) of the terpolymer. To understand the formation mechanisms of microspheres of the present study is the subject of future work.

2.3.8 Crystals precipitated in the presence of terpolymer with poly-L-aspartic acid

When the synthetic poly (AM-NaAMPS-NVP) with monomer compositions of 80:10:10 mole % was used in presence of poly-L-aspartic acid, the crystallization of CaCO₃ resulted in a hollow needle-like or plate-like morphology with rough surfaces is found (See Figure 2.18 & Figure 2.21: 2a, 2b). A closer examination of these elongated hollow crystals indicated that it is an array of columnar rods. The rods had a length of 5-10 µm and were highly oriented parallel to each other. XRD pattern of the collected crystals showed strong diffraction maxima at 2θ ~ 32.8° that correspond to aragonite modification. FTIR
analysis (Figure 2.22c) confirmed the presence of aragonite at 850 cm\(^{-1}\) which is the characteristic peak (Pai et al. 2004).

![Aragonite Hollow Crystals](image)

**Figure 2.18. SEM micrographs of aragonite hollow crystals.** CaCO\(_3\) mineralization in the presence of synthetic poly (AM-NaAMPS-NVP) with monomer compositions of 80:10:10 mole % was used in presence of poly-L-aspartic acid. Source: Pai, et al. *Langmuir* 2004, 20, 3123-3128.

In the control experiments in which no polymer/poly-Asp were present, rhombohedral crystals characteristic of calcite were produced as shown in Figure 2.19A & Figure 2.21: 1a, 1b. Also we can see when poly (AM-NaAMPS-NVP) was used as an additive in the absence of poly-Asp; the crystallization of CaCO\(_3\) resulted in stacked crystal aggregates with a mean size of 10 µm (Figure 2.19B); this suggests that the necessity of poly-Asp for controlling the crystal growth in combination with terpolymer.
Figure 2.19. SEM micrographs of CaCO₃ crystals. CaCO₃ mineralization in the (A) absence of poly-Asp and synthetic polymer, (B) in the presence of synthetic polymer without poly-Asp. Source: Pai, et al. Langmuir 2004, 20, 3123-3128.

AFM is capable of nanometer resolution on crystal surfaces (Quate et al. 1994 and Wiesendanger et al. 1994) and excels at imaging a wide variety of biomolecules (Shao et al. 1996). AFM can also image under liquids, enabling process of crystal growth to be studied in situ (Bosbach et al. 1995). I therefore have used AFM to examine the effects of polymer/poly-Asp on a growing crystal surface in situ. Figure 2.20 (1a, 1b) shows the typical tapping mode AFM images of terpolymer surface. Here the surface is smooth ~ 15 nm with layered structure indicating some phase separation.

After entrapment of Poly-Asp in the terpolymer in Figure 2.20 (2a, 2b), has enabled us to visualize in situ the interaction of Poly-Asp on terpolymer resulted in sponge like structure with surface roughness ~300 nm. Figure 2.20 (2a, 2b) and Figure 2.21 (1a, 1b and 2a, 2b) are at higher magnifications, however, the AFM revealed, the adsorbates, that are not visible otherwise. These images make possible, direct, visual observation of molecules that are adsorbed on crystal surfaces. If the adsorbates are tightly bound on a surface that is stable enough so
that the AFM probe does not displace the molecules.

Figure 2.20. AFM to examine the effects of polymer /poly-Asp.
Representative tapping mode AFM images of (a) height data, (b) phase data (1a, 1b) synthetic water-soluble terpolymer, and (2a, 2b) synthetic water-soluble terpolymer with poly-Asp. Source: Pai, et al. *Langmuir* 2004, 20, 3123-3128.

The crystal grown in the presence of terpolymer with poly-Asp has the typical morphology of needle like or plate like aragonite (Figure 2.18 & Figure 2.21 2a, 2b).
Figure 2.21. **AFM image of aragonite crystals.** Representative tapping mode AFM images of (a) height data, (b) phase data; CaCO₃ crystals grown (1a, 1b) in the absence of synthetic water-soluble terpolymer and poly-Asp, and (2a, 2b) in the presence of synthetic water-soluble terpolymer and poly-Asp. Source: Pai, et al. *Langmuir* 2004, 20, 3123-3128.

The generation of more nucleation sites in the Poly-Asp may be responsible for the selective nucleation of aragonite crystals. Helmut Cölfen et al (Milos et al. 1998) shown that the crystallization inhibition efficiency of the double hydrophilic block copolymer is about 3-4 times
higher, due to the incorporation of Poly-Asp. We have not yet systematically studied the kinetics as a function of polymer/poly-Asp concentration and pH, however, under specific experimental conditions we have observed, the growth of hollow crystals of aragonite. The stabilization of hollow crystals of aragonite on terpolymer in the presence of poly-L-aspartic acid could be due to the change in the chemical environment at the terpolymer surface. This is supported by AFM results. It is known that formation of the stabilized crystal phase requires strong Ca – O interaction with the substrate surface (Ueyama et al. 1998) or additives. Poly-Asp molecules become part of the crystal lattice in the sense that anionic carbonyl groups of aspartate residues substitute for carbonate ions of the lattice. This enhances the binding energy between the Polymer/Poly-Asp and the crystal surface. Poly-Asp have very strong binding energies when stereo chemically bound to aragonite, particularly if they become incorporated into the crystal lattice. This was confirmed by FTIR (Figure 2.22).

It is likely that the presence of poly-L-aspartic acid increases the number of polar functional groups, which are known to interact strongly with polar groups of the terpolymer forming a complex organic matrix. This may result in the selective nucleation of hollow crystals of aragonite. Another explanation for the growth of these hollow plates like aragonite crystals can be the initial formation of an unstable CaCO₃ modification, such as amorphous CaCO₃. With time, amorphous CaCO₃ will be transformed to the more stable modifications especially in the presence of moisture. Gutjahr, et al (Gutjahr et al. 1996) explained this transformation clearly by the Ostwald step rule. Under standard conditions, the vaterite phase
readily transforms into the stable aragonite or calcite phase in the presence of water through a solvent-mediated mechanism (Nassrallah-Aboukais et al. 1998). This process involves dissolution of vaterite crystals followed by nucleation of the calcite phase and generally takes place within 80h. Such transformation could probably be suppressed by the combined effect of terpolymer/poly-Asp. The structural transformation form vaterite to aragonite may leads to hollow crystals.

Figure 2.22 shows the FTIR spectra recorded from the water soluble terpolymer (curve a), the CaCO₃ crystals grown in the presence of polymer without Poly-Asp (curve b), the CaCO₃ crystals grown in the presence of polymer with Poly-Asp (curve c), the CaCO₃ crystals grown in the absence of polymer and Poly-Asp (curve d). Prominent absorption bands are seen at 1377, 1460, 1545, 1633, 1663, 2855, 2922, 3429 cm⁻¹ (Figure 2.22a) in the case of the terpolymer sample. The band at 1377 cm⁻¹ is assigned to the S = O stretching vibration of the sulfonate group present in the terpolymer. The band at 1545, 1633 and 1663 cm⁻¹ is due to carbonyl stretch vibrations of AMPS, NVP and AM respectively. Amide unit of the terpolymer comes at 3429 cm⁻¹. Methyl group of AMPS unit comes at 1460 cm⁻¹ and the two bands at 2855 and 2922 cm⁻¹ have been assigned to the methylene symmetric and antisymmetric stretching vibrations respectively, in the hydrocarbon chains (Figure 2.22a). The methylene antisymmetric and symmetric vibrations have reduced marginally in intensity following Ca⁡⁺⁺ incorporation and we can see that strong infrared absorption bands at 713 and 877 which is the characteristic of the calcite polymorph (Figure 2.22b).
Figure 2.22. FTIR Results. FTIR spectra recorded from the water-soluble terpolymer (curve d), the CaCO₃ crystals grown in the presence of polymer without poly-Asp (curve c), the CaCO₃ crystals grown in the presence of polymer with poly-Asp (curve b), and the CaCO₃ crystals grown in the absence of polymer and poly-Asp (curve a). Source: Pai, et al. *Langmuir* 2004, 20, 3123-3128.

The almost complete disappearance of the methylene antisymmetric and symmetric vibration bands after aragonite formation (Figure 2.22c) indicates considerable reorganization in the terpolymer chemical structure, possibly due to the randomization of bioorganic macromolecular chain orientation around the aragonite crystals. After entrapment of poly-L-aspartic acid in the polymer, the absorption band at 1377 cm⁻¹ has shifted to 1195 cm⁻¹ (Figure 2.22c) clearly indicating that the protein have complexes with the sulfonate group of the terpolymer. Also the presence of absorption bands at 2512
and 2927 cm\(^{-1}\) of the carboxyl group and band at 1685 cm\(^{-1}\) of the amide group indicate the presence of Poly-Asp in the polymeric substrate. The strong infrared absorption bands at 850 cm\(^{-1}\) is the characteristic of the aragonite polymorph. A small absorption band at 877 cm\(^{-1}\) characteristic to the calcite polymorph indicates that there is some transformation of aragonite into the calcite modification.

2.3.9 Formation of different morphologies of calcium carbonate: Role of Poly (AM-NaAMPS-SA) under the control of various additives

In the control experiment in which no low molecular weight additives or polymer were present, different morphologies like stacked rhombohedral aggregates, spherical and hemispherical crystal aggregates were produced in the size range between 3 – 10 µm as shown in Figure 2.23A-D; this suggest that the absence of additives has no effect in controlling the size, morphologies, nucleation and growth rate. FTIR analysis shows that the sample consist mixture of calcite, vaterite and aragonite polymorphs (Figure 2.25 C-1), whereas XRD shows only calcite and vaterite modifications (Figure 2.34 C-1).

CaCO\(_3\) grown in presence of the polymer without additives are shown in (Figure 2.24A). It is observed that these aggregates of spherical morphology with diameters between 10 and 20 µm are formed.
Figure 2.23. SEM micrographs of CaCO$_3$ crystals. Representative SEM images of the CaCO$_3$ crystal aggregates grown in the absences of polymer and low molecular weight additives.

Figure 2.24. SEM micrographs of CaCO$_3$ crystals. Representative SEM images of the CaCO$_3$ crystal aggregates grown in the presence of polymer without low molecular weight additives.

The spheres consist of rhombohedral sub crystals, with sizes in the range between 500 - 800 nm. FTIR, XRD and Raman analysis show clearly that the crystalline phase consist only of the calcite polymorph (Figure 2.25 C-2, 2.35e, 2.36a respectively).
Precipitation experiments conducted with or without the terpolymer with three different additives [L-glutamine, N-acetyl-L-glutamic acid (monomers), and glutathione (tri-peptide)] indicate that the additives in combination with terpolymer having a more pronounced effect in the morphologies of calcium carbonate aggregates than the additives alone (See Figure 2.26, 2.28, 2.30 & 2.32).

Precipitation in the presence of L-glutamine results in spheres with diameters in the range between 9 -12 µm (Figure 2.26A, B). The spheres consist of median groove with smooth surface and capped with

---

12 Catherine, et al. (1977); #Kennedy, et al. (1987); ‡Weir, et al. (1961); †White, et al. (1974)
stacked rhombohedral spherical aggregates and also found few single rhombohedral spherical aggregates in the size range between 5 -10 µm. FTIR analysis (Figure 2.27C-Exp-2) shows clearly that in these spherical aggregates both the calcite and vaterite polymorph are present. But latter is the predominant form and confirmed by XRD analysis (Figure 2.34C-Exp-2).

Figure 2.26. SEM micrographs of CaCO3 crystals. Representative SEM images of the CaCO3 crystal aggregates grown in the presence of (A & B) L-Glutamine without Polymer and (C & D) in combination with L-Glutamine and polymer

Whereas L-glutamine in combination with the terpolymer resulted in spheres with diameters in the range of 1 -5 µm (Figure 2.26C, D). Like in the earlier case the spheres consist of sub crystals, but they were tiny spheres, which have sizes in the range of 30-100 nm. I assumed that the reduced size of the spheres is an indication for a
strong interaction between the carboxylic acid groups of L-glutamine and the crystallizing CaCO₃ have shown that this effectively suppresses crystal growth. XRD suggests that both calcite and vaterite are present but vaterite in the predominant form (Figure 2.35c). This result is confirmed by Raman spectroscopy (Figure 2.37A).

### Figure 2.27. Summary of the experimental results. Left) FTIR analysis shows summary of the experimental results of the nucleation of CaCO₃ modifications and right) IR spectra for the corresponding analysis: (C-Exp-2) in the presence of L-glutamine and (Exp-2) in the presence of L-glutamine with poly (AM-NaAMPS-SA). IR peaks from poly (AM-NaAMPS-SA), see Table 2.1.

The original spectrum (Figure 2.37A1) shows a broad band ranging from 200 – 400 cm⁻¹ and two small peaks at 711 cm⁻¹ and 740 cm⁻¹, characteristic for calcite and vaterite, respectively. To prove the presence of vaterite, the calcite fraction weighed with 75 % was subtracted from the original spectrum (see Table 2.2). The remaining spectrum (Figure 2.37A2) clearly reveals two double peaks at 268 / 301 cm⁻¹ and 1074 / 1089 cm⁻¹ as well as small peak at 740 cm⁻¹. These peaks are characteristic for the vaterite polymorph. A shoulder appearing on

---

13 †Kennedy, et al. (1987); ‡Weir, et al. (1961); †White, et al. (1974)
209 cm\(^{-1}\) indicates that aragonite is also present in low amounts. The corresponding IR spectrum (Figure 2.27Exp-2) shows sharp bands at 879/750 and 673 cm\(^{-1}\), characteristic for vaterite; providing further evidence for the presence of vaterite.

Crystallization experiment carried out with N-acetyl-L-glutamic acid, results in the formation of twinned hemisphere morphology in the size range between 3-10µm (Figure 2.28A-D). The twinned hemisphere consists of tiny spherical sub crystal, which has size in the range of 60-10nm. Several dumbbell-like and spindle-like rhombohedral crystal aggregates were formed in the size range between 4 - 6 µm (Figure 2.28A, B). FTIR analysis (Figure 2.29C-Exp-3) shows the presence of mixture of calcite, aragonite and vaterite polymorph, whereas XRD analysis shows only calcite and vaterite modifications (Figure 2.34C-Exp-3).

Terpolymer preadsorbed by N-acetyl-L-glutamic acid, results in the formation of twinned hemisphere morphology (See Figure 2.28E, F). The dimensions of the hemispheres are in the range of 6 - 10 µm. A closer examination of these twinned hemisphere crystals indicated that the sub crystals are rhombohedral in shape. The size of the sub-crystals ranges between 200 - 300nm. Many authors (McCauley et al. 1974, Fernandez-Diaz et al. 1996 and Grassmann et al. 2002) observed similar CaCO\(_3\) morphology grown in silica and gelatin, respectively. The XRD pattern (Figure 2.35b) exhibits only sharp calcite peaks. However, the corresponding Raman spectra (Figure 2.37B1) depicts that beside calcite also small amounts of amorphous calcium carbonate (ACC) is present because a broad band ranging from 200 – 390 cm\(^{-1}\) appears in the spectrum after calcite subtraction (Figure 2.37B2).
In addition small peaks characteristic of the vaterite polymorph are observed (Table 2.2). FTIR supports these findings (Figure 2.29Exp-3). In the FTIR spectrum of the calcium carbonate precipitate a strong band at 879/714 cm\(^{-1}\) has been observed, which is characteristic for well-ordered calcite, and an absorption peak at 866 / 873 cm\(^{-1}\) appears, which is characteristic for stable ACC (Addadi et al. 2003).
CaCO₃ Crystal Modifications Using Water-soluble Polymers Based on AMPS Monomer

Figure 2.29. Summary of the experimental results. Left) FTIR analysis shows summary of the experimental results of the nucleation of CaCO₃ modifications and right) IR spectra for the corresponding analysis: (C-Exp-3) in the presence of N-acetyl-L-glutamic acid and (Exp-3) in the presence of N-acetyl-L-glutamic acid with poly (AM-NaAMPS-SA). IR peaks from poly (AM-NaAMPS-SA), see Table 2.1.

In presence of glutathione, selective nucleations of CaCO₃ with twinned hemisphere morphology were formed in the size range between 5-20 µm (Figure 2.30A-D). Closer examination of these twinned hemispheres shows smooth surface with tiny granular subcrystals in the size range between 50–80 nm. FTIR analysis shows the presence of vaterite and ACC (Figure 2.31C-Exp-4), whereas XRD shows only vaterite (Figure 2.34 C-Exp-4).

---

14 ‡Weir, et al. (1961); †White, et al. (1974)
Figure 2.30. SEM micrographs of CaCO₃ crystals. Representative SEM images of the CaCO₃ crystal aggregates grown in the presence of (A - D) Glutathione without Polymer and (E & F) in combination with Glutathione and polymer.

Whereas Glutathione preadsorbed terpolymer induced a similar morphology like the sample showed before (Figure 2.30C). Here the surface was smooth (Figure 2.30E) as similar in the case before. The sub crystals are irregular tiny crystals in the size range 50 - 100nm (Figure 2.30F). This sample contains both calcite and vaterite polymorphs as shown by XRD patterns (Figure 2.35a) and confirmed by FTIR and Raman analysis (Figure 2.31Exp-4 and Figure 2.37C2). The IR (Figure 2.31Exp-4, Table 2.1) and Raman analysis indicates also the presence of
small amounts of ACC (see Table 2.2). The discrepancy between XRD and spectroscopy methods might be due to the fact that the XRD will not detect the polymorph if the sample contains less than ca.10 mass% of the according modification.

![Table 2.2](image)

<table>
<thead>
<tr>
<th>Modification</th>
<th>Reference peak cm⁻¹</th>
<th>FTIR peaks from experimental data cm⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcite</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>2975</td>
<td>2975</td>
</tr>
<tr>
<td></td>
<td>2931</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2815</td>
<td>2815</td>
</tr>
<tr>
<td></td>
<td>1409</td>
<td>1409</td>
</tr>
<tr>
<td></td>
<td>877 / 714</td>
<td>877 / 714</td>
</tr>
<tr>
<td>Vaterite</td>
<td>2250</td>
<td>2250</td>
</tr>
<tr>
<td></td>
<td>1936</td>
<td>1936</td>
</tr>
<tr>
<td></td>
<td>1409</td>
<td>1409</td>
</tr>
<tr>
<td></td>
<td>1409 / 1099</td>
<td>1409 / 1099</td>
</tr>
<tr>
<td></td>
<td>877 / 740 / 608</td>
<td>877 / 740 / 608</td>
</tr>
<tr>
<td></td>
<td>850</td>
<td>850</td>
</tr>
<tr>
<td>ACC</td>
<td>866 / 874</td>
<td>866 / 874</td>
</tr>
<tr>
<td></td>
<td>760 / 700</td>
<td>760 / 700</td>
</tr>
<tr>
<td></td>
<td>720</td>
<td>720</td>
</tr>
</tbody>
</table>

Figure 2.31. Summary of the experimental results. Left) FTIR analysis shows summary of the experimental results of the nucleation of CaCO₃ modifications and right) IR spectra for the corresponding analysis: (C-Exp-4) in the presence of glutathione and (Exp-4) in the presence of glutathione with poly (AM-NaAMPS-SA). IR peaks from poly (AM-NaAMPS-SA), see Table 2.1.

Falini et al (Falini et al. 1998) have shown that magnesium ions have a strong influence towards aragonite formation. Because of this, precipitation was carried out with 0.1 M magnesium chloride. The resulting precipitates had four different morphologies: spindle-like, dumbbell-like, cross-like and mulberry like. Their sizes were in the range between 7-9 µm (Figure 2.32a A-D). Sub crystals are stacked rhombohedral aggregates with rough surface. FTIR shows mixtures of

---

15 †Catherine, et al. (1977); ‡Kennedy, et al. (1987); ‡Weir, et al. (1961); †White, et al. (1974)
calcite, aragonite and ACC modifications (Figure 2.33C-Exp-1), whereas XRD shows only calcite (Figure 2.34 C-Exp-1).

Whereas magnesium chloride in combination with terpolymer/0.1 M magnesium chloride (Figure 2.32b A-D) show aggregates with sizes ranging from 4 - 6 µm, which form four different shapes as in the case before: spindle-like, dumbbell-like\(^{16}\), cross-like and mulberry like\(^{17}\). Spherical shaped sub-crystals with a diameter in the range of 100 nm appear on the surface. The XRD pattern recorded shows the presence of only calcite crystals (Figure 2.35d). Nevertheless, in the Raman spectrum a shoulder at 200 cm\(^{-1}\) appears (Figure 2.37D1). This indicates the presence of aragonite. After subtracting the calcite fraction aragonite peaks at 209, 705 and 1087 cm\(^{-1}\) can clearly be seen in the remaining spectrum (Figure 2.37D2). The presence of aragonite was further verified by FTIR analysis and observed the characteristic absorption bands at 860/713/707 cm\(^{-1}\)(Figure 2.33Exp-1). In addition to this several peaks for ACC were observed in IR analysis (Figure 2.33Exp-1).

\(^{16}\) Cölfen and coworkers (Cölfen, et al. 2001) were also obtained similar morphology by using double-hydrophilic block copolymer PEG-b-PMAA as an additive.

\(^{17}\) Grassmann and coworkers (Grassmann et al. 2002) observed similar CaCO\(_3\) morphology grown within gelatin and artificial p-AAm gel matrices in the presence of p-L-Asp.
Figure 2.32. **SEM micrographs of CaCO$_3$ crystals.** Representative SEM images of the CaCO$_3$ crystal aggregates grown in the presence of (Figure 2.32a A - D) magnesium without Polymer and (Figure 2.32b A-D) in combination with magnesium and polymer.
As pointed out earlier, many studies on the influence of various cations were reported in literature and it is confirmed that Mg$^{2+}$ ions exert a significant effect on CaCO$_3$ crystallization behavior.

<table>
<thead>
<tr>
<th>Modifications</th>
<th>Reference peak cm$^{-1}$</th>
<th>FTIR peaks from experimental data cm$^{-1}$</th>
<th>C-Exp-1</th>
<th>Exp-1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcite$^1$</td>
<td>1677 1417 1393</td>
<td>2525</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>877 / 714 / 708</td>
<td>860 / 713 / 709</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aragonite$^4$</td>
<td>1677 1476 1476</td>
<td>1411</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>864 / 873 864 / 873</td>
<td>864 / 867</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ACC$^6$</td>
<td>719 719 719 709</td>
<td>719</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

![Figure 2.33. Summary of the experimental results. Left) FTIR analysis shows summary of the experimental results of the nucleation of CaCO$_3$ modifications and right) IR spectra$^{18}$ for the corresponding analysis: (C-Exp-1) in the presence of magnesium and (Exp-1) in the presence of magnesium with poly (AM-NaAMPS-SA). IR peaks from poly (AM-NaAMPS-SA), see Table 2.1.](image)

Adding a sufficient amount of Mg$^{2+}$ brings about precipitation of aragonite rather than the thermodynamically stable calcite modification. The shifts observed in FTIR spectrum (compare Figure 2.25C-2 and Figure 2.33C-Exp-1, Exp-1), in our opinion, are attributed to either compositional inhomogeneity or disorder caused by magnesium substitution. White and coworkers (White et al. 1974) also reported similar observations.

---

$^{18}$ $^1$Catherine, et al. (1977); $^2$Kennedy, et al. (1987); $^3$Weir, et al. (1961); $^4$White, et al. (1974)
Aragonite phase displays an additional peak around 1083 cm\(^{-1}\) (Figure 2.33C-Exp-1, Exp-1), which is in agreement with the data reported by Aizenberg and coworkers (Aizenberg et al. 1996). The band broadening in Raman spectrum (Figure 2.37D2), which is in agreements with Bischoff and coworkers (Bischoff et al. 1985), supports the above assumption.

Like in previous studies of organic template driven crystallization of inorganic salts, the results obtained in the present work indicate that the crystalline structures and the shape of the aggregates of CaCO\(_3\) depends on the nature of additives and the polymer employed. The stabilization of crystal modifications and morphologies could be due to the change in the chemical environment at the terpolymer surface when they come in contact with different salts solutions and additives. Atomic force microscopy (AFM) has been used to examine the variation of the terpolymer [Poly (AM-NaAMPS-SA)] conformation, when they come in contact with several low molecular weight additives.

Poly (AM-NaAMPS-SA) posses ionizable groups, which dissociate in aqueous media to give negatively charged polymer chains and positively charged counterions. The repulsion between charged monomers tends to expand the polymer chain. The high charge density on the polymer backbone produces a high electrostatic potential around it and a fraction of counterions are consequently located in the immediate vicinity of the polymer chain leading to phase separation at low salt concentration, if counterions are multivalent (Michaeli et al. 1960, Ikegami et al. 1962, Kimizuka et al. 1967).
Figure 2.34. Summary of XRD analysis. XRD analysis shows different calcium carbonate modifications obtained by the mineralization of CaCO$_3$ using various additives.
Figure 2.35. **Summary of XRD analysis.** XRD spectra recorded from the CaCO₃ in the presence of polymer with (a) Glutathione, (b) N-Acetyl-L-glutamic acid, (c) L-Glutamine, (d) magnesium, and (e) polymer without additives.

Figure 2.36. **Raman analysis.** Raman spectra recorded from (a) CaCO₃ precipitated in presence of polymer without additives and (b) the pure synthetic calcite (reference spectrum).
Figure 2.37. **Summary of Raman analysis.** After subtracting the calcite spectra, the remaining Raman spectra shows CaCO₃ precipitated in presence of polymer with (A) L-Glutamine, (B) N-acetyl-L-glutamic acid, (C) Glutathione and (D) Magnesium. (A = aragonite, V = Vaterite and ACC = amorphous calcium carbonate) (1) = Before Calcite subtraction and (2) = After Calcite subtraction.
**Table 2.1. FTIR peak table for terpolymer.** FTIR table shows presence of polymer in the samples: (Polymer) IR peaks from poly (AM-NaAMPS-SA) and the nucleation of CaCO₃ modifications: (C-2) in the presence of poly (AM-NaAMPS-SA) without additives, (Exp-1) in the presence of poly (AM-NaAMPS-SA) with magnesium, (Exp-2) in the presence of poly (AM-NaAMPS-SA) with L-glutamine, (Exp-3) in the presence of poly (AM-NaAMPS-SA) with N-acetyl-L-glutamic acid, (Exp-4) in the presence of poly (AM-NaAMPS-SA) with glutathione.
CaCO₃ Crystal Modifications Using Water-soluble Polymers Based on AMPS Monomer

<table>
<thead>
<tr>
<th></th>
<th>Shifts cm⁻¹</th>
<th>C-2 cm⁻¹</th>
<th>Exp-1 cm⁻¹</th>
<th>Exp-2 cm⁻¹</th>
<th>Exp-3 cm⁻¹</th>
<th>Exp-4 cm⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcite</td>
<td>279</td>
<td>279</td>
<td>279</td>
<td>279</td>
<td>279</td>
<td>279</td>
</tr>
<tr>
<td></td>
<td>711</td>
<td>711</td>
<td>711</td>
<td>711</td>
<td>711</td>
<td>711</td>
</tr>
<tr>
<td></td>
<td>1087</td>
<td>1084</td>
<td>1084</td>
<td>1084</td>
<td>1084</td>
<td>1084</td>
</tr>
<tr>
<td>Calcite fraction %</td>
<td>100</td>
<td>90</td>
<td>65</td>
<td>90</td>
<td>85</td>
<td></td>
</tr>
<tr>
<td>Aragonite</td>
<td>209</td>
<td>209</td>
<td>209</td>
<td>209</td>
<td>209</td>
<td>209</td>
</tr>
<tr>
<td></td>
<td>700 / 705</td>
<td>701 / 705</td>
<td>701 / 705</td>
<td>701 / 705</td>
<td>701 / 705</td>
<td>701 / 705</td>
</tr>
<tr>
<td></td>
<td>1087</td>
<td>1084</td>
<td>1084</td>
<td>1084</td>
<td>1084</td>
<td>1084</td>
</tr>
<tr>
<td>Vaterite</td>
<td>268 / 301</td>
<td>269 / 300</td>
<td>269 / 300</td>
<td>269 / 300</td>
<td>269 / 300</td>
<td>269 / 300</td>
</tr>
<tr>
<td></td>
<td>738 / 750</td>
<td>741</td>
<td>741</td>
<td>741</td>
<td>741</td>
<td>741</td>
</tr>
<tr>
<td></td>
<td>1074 / 1089</td>
<td>1071 / 1094</td>
<td>1071 / 1094</td>
<td>1071 / 1094</td>
<td>1071 / 1094</td>
<td>1071 / 1094</td>
</tr>
<tr>
<td>ACC</td>
<td>227</td>
<td>234</td>
<td>234</td>
<td>234</td>
<td>234</td>
<td>234</td>
</tr>
<tr>
<td></td>
<td>725</td>
<td>1081</td>
<td>1081</td>
<td>1081</td>
<td>1081</td>
<td>1081</td>
</tr>
</tbody>
</table>

Table 2.2. Raman peak table. Raman table shows different calcium carbonate modifications obtained by the mineralization of CaCO₃: (C-2) in the presence of poly (AM-NaAMPS-SA) without additives, (Exp-1) in the presence of poly (AM-NaAMPS-SA) with magnesium, (Exp-2) in the presence of poly (AM-NaAMPS-SA) with L-glutamine, (Exp-3) in the presence of poly (AM-NaAMPS-SA) with N-acetyl-L-glutamic acid, (Exp-4) in the presence of poly (AM-NaAMPS-SA) with glutathione.

The stability of the polyelectrolyte solution depends on the counterion valency and the characteristics of the polymer: nature of backbone (hydrophobic, hydrophilic, rigid and flexible), distance between charged groups and the nature of charged groups (carboxylate, sulfonate, carbonyl and amide). AFM imaging on terpolymer surface, in presence of divalent cations and several low molecular weight additive containing charged groups, were established. Figure 2.38 shows the typical tapping mode AFM images.
of terpolymer surface. Here we could see some phase separation: a lower spherical area and an upper cloudy area.

**Figure 2.38.** AFM analysis. Representative tapping mode AFM images of (A) height data, (B) phase data: (1A & 1B) synthetic water soluble terpolymer.

In presence of different salt solutions, we could be able to see that, the variation of the terpolymer conformation (Figure 2.39). Entrapment of L-glutamine, N-acetyl-L-glutamic acid and glutathione in the terpolymer, (Figure 2.40) has enabled us to visualize in situ the interaction of low molecular weight additives on terpolymer, resulting in compaction of macromolecular coils caused by deterioration of thermodynamic quality of a solvent. Due to this reason at very high salt concentration polymers can loose their solubility and precipitate from solution. These images make possible direct, visual observation of micro-environmental changes brought about by the adsorption of additives (acidic peptides / simple aminoacids / magnesium ions) play a significant role in inducing the deposition of different CaCO₃ crystal modifications.
Figure 2.39. AFM analysis. Representative tapping mode AFM images of (A) height data, (B) phase data: Synthetic water-soluble terpolymer (1A & 1B) with Calcium Chloride, (2A & 2B) with Magnesium chloride and (1A & 1B) with Calcium & Magnesium chlorides.
Figure 2.40. AFM analysis. Representative tapping mode AFM images of (A) height data, (B) phase data: Synthetic water-soluble terpolymer (1A & 1B) with L-Glutamine, (2A & 2B) with N-Acetyl-L-glutamic acid, (3A & 3B) with Glutathione.
2.4 Conclusion

The observed aggregate shape and the selectivity for the crystal modification can be attributed to the co-operative effect of pre-adsorbed additives and the functionalities (amide, carboxylic, sulphonic acid) of the terpolymer. The interaction between various functional groups and ionic species present in CaCO$_3$ and stereochemical matching between the oriented functional groups and the crystal plane are fundamentally responsible for the stabilization of specific aggregates. Nucleation of the calcite {110} faces is favoured by carboxylate anions because the bidentate motif mimics the carbonate stereochemistry exposed on this crystal surface. On the other hand, the nucleation of the {001} face is induced by sulfate head groups because the tridentate arrangement stimulates the oxygen positions of carbonate anions lying parallel to this crystal surface (Heywood et al. 1994). Thus it appears most likely that the initiation and nucleation of various forms of crystal modifications on the terpolymer employed occurs through binding of calcium ions at the ionizable carboxylic and sulphonic groups.

Summary of FTIR analysis shows that in all samples methylene symmetric and antisymmetric vibrations have reduced marginally in intensity following Ca$^{2+}$ incorporation. This indicates considerable reorganization in the polymer, possibly due to randomization of hydrocarbon chain orientation around the CaCO$_3$ crystals. This is supported by AFM results. In some cases, we could be able to see from IR spectra almost complete disappearance of the methylene symmetric and antisymmetric vibration bands after CaCO$_3$ nucleation which indicates that the considerable reorganization in the polymer chemical
structure is possibly due to the randomization of several low molecular weight additives or macromolecular chain orientation around the CaCO$_3$ crystals. After entrapment of additive, the absorption band for the sulphate group from polymer at 1046 cm$^{-1}$ has shifted (Table 2.1) clearly indicating that the additives complexes the sulfonate group of the terpolymer.
Chapter 3

Miniemulsion-based Synthesis of Calcium Carbonate

In this chapter, I will describe a versatile method for the nucleation of calcium carbonate aggregates with different morphologies and textures fabricated by the miniemulsion technique. Inverse miniemulsions can be prepared by emulsifying an aqueous inorganic salt solution in a continuous oil phase. The solution is dispersed in the continuous phase by means of an adequate amount of surfactant by fast stirring and the efficient use of ultrasonication. Here small droplets in the size range of 50-500 nm with sufficient stability are created. For the preparation one inverse miniemulsion consisting of \( \text{Ca}^{2+} \) ions containing droplets and another miniemulsion consisting of \( \text{CO}_3^{2-} \) ions containing droplets are prepared. Adding these two miniemulsions while applying high shear lead to the \( \text{CaCO}_3 \) nanoparticles. The morphology of the crystals can be tuned effectively by various parameters. This kind of calcium carbonate modifications has a bright future for various applications.

3.1 Introduction

Calcium carbonate (\( \text{CaCO}_3 \)) has been widely used as fillers for papermaking, coatings, plastics and agriculture. Its primary function in the paper industry is to reduce furnish costs and increase the brightness of paper, while still being inexpensive. The are some main functions of \( \text{CaCO}_3 \) as filler (Scott, W. E. 1996): improving printing quality by changing the smoothness, show through, ink absorption, formation and sheet structure, appearance properties, dimensional
stability, texture and feel. In the plastics industry it is used as filler in polymer composites such as plasticized and rigid PVC, unsaturated polyesters, polypropylene and polyethylene [http://www.ima-eu.org]. In coatings, calcium carbonate is used as the main extender. The opacity of coatings is influenced by fineness and particle size distribution. Also, calcium carbonate enhances properties of the coating such as: weather resistance, anti-corrosion, rheological properties, low abrasiveness. In water based coating calcium carbonate also reduces the drying time [http://www.ima-eu.org]. Calcium carbonate is also used in agriculture as a fertilizer. It is used to stabilize the pH of the soil. It is also used at times as a calcium supplement in animal feed stock.

CaCO$_3$ nanoparticles (<100 nm) have shown many unique properties compared to regular CaCO$_3$ particles (>3 µm) (Bates et al. 1985). Studies on the effects of precipitated calcium carbonate fillers in sealants and PVC materials have been made. It has been indicated that fatty acid-treated precipitated calcium carbonate of particle size less than 100 nm is particularly useful for filling sealants. Studies on the impact fracture energy of mineral-filled polypropylene and copolymer with and without calcium carbonate fillers showed that nanometer fillers increased the stiffness of both the homopolymer and copolymer (Chen et al. 1989). Kovacevic and coworkers (Kovacevic et al. 2002) found calcium carbonate nanocomposites exhibit unique and improved properties in polymer composites appeared. In poly (vinyl acetate) (PVAc) matrix, the morphology of the composite was found to be dependent on the filler particle size. The nanoparticles form ‘net-like’ dispersion in the matrix, whereas the particles in the micron scale formed ‘islands’. Qui and coworkers (Qiu et al. 2000) studied the
application of CaCO₃ nanoparticles as additives in lubricating oils. It was found that CaCO₃ nanoparticles exhibited good load-carrying capacity, antiwear and friction-reducing properties.

Thus far, there is little evidence of an optimized method to control size and morphology of CaCO₃ particles. Current methods used make it difficult to predict particle size and morphology and require large amounts of energy. Studies have been done on the use of liquid emulsion membrane (LEM) for the production of nanoparticles (Hirai et al. 2000), such as calcium phosphate fine particles. However, these studies used surface chemistry such as surfactant adsorption and phase stability to control the particle size and shape, which is different from the route using mass restriction and kinetic control for synthesis.

3.1.1 Methods of Synthesis of Nanoparticles

The synthesis of organized extended structures based on the assembly of nanostructured building blocks is currently recognized as an important theme at the nano–micro interface. A number of strategies have been developed to spatially pattern and control higher-order organization, including chemical and microfabrication methods, (Yang et al. 2001) and controlled deposition of nanoparticle-based superlattices using solvent evaporation, (Wang et al. 1998) molecular cross-linking (Brust et al. 1995) or programmed recognition (Mirkin et al. 1996). A nanophase material synthesized by gas evaporation methods (Gonzalez et al. 2001), was introduced by Granqvist and Burman. However, thermal evaporation is a known limitation to this method for metals and intermetallic compounds. This was later overcome by Hahn and Averback (Hahn et al. 1990), by substituting the thermal evaporation source with a sputtering source, thus enabling the
synthesis of nanoparticles. However, the size of the particles depended on pressure of Ar in the operating chamber. Small changes in pressure would change the particle size.

Wang and coworkers (Wang et al. 2001) synthesized nanometer size CaCO₃ (15-40 nm) using a lime suspension in a rotating packed bed reactor and had a very narrow distribution. It was reported that the most important stage for controlling the carbonation rate was the absorption of CO₂. However, it was latter discovered to be controlled by dissolution of Ca (OH)₂. This method is also known as “high gravity multiphase reactive precipitation.” The method required a high acceleration centrifuge to create the high gravity above the gravity of the earth. This required expensive synthesis equipment. However they also reported that the shape and morphology could be controlled.

Tsuzuki and coworkers (Tsuzuki et al. 2000) synthesized calcium carbonate nanoparticles using a mechanochemical reaction followed by heat treatment. A solid-state displacement reaction would occur during mechanical milling of the reaction powder mixture. The heat treatment ensured completion of the reaction. This limited the morphology of the particle to calcite, and had high energy consumption. Mechanical milling causes irregularities in particle shape and distribution.

Alternatively, recent studies have demonstrated that complex macroscopic structures consisting of self-organized surfactant–inorganic nanofilaments of BaSO₄, (Hopwood et al. 1997 and Li et al. 2000) BaCrO₄, (Li et al. 1999) BaCO₃, (Qi et al. 1997) CaSO₄, (Rees et al. 1999) or CaCO₃ (Li et al. 2002, 2003) can be synthesized in water-in-oil microemulsions by emergent processes involving nucleation and growth within aggregates of surfactant-coated amorphous inorganic nanoparticles. The amorphous nanoparticles slowly crystallize within
the colloidal aggregates, and because this process is strongly coupled with rearrangement of the surface adsorbed organic molecules, the resulting nanocrystals exhibit time and scale-dependent structures and morphologies (Cölfen et al. 2003).

Recently Viravaidya et al. (2004) reported the spontaneous formation of novel complex architectures of the thermodynamically stable calcium carbonate polymorph, calcite, by addition of sodium carbonate-containing Sodium bis (2-ethylhexyl) sulfosuccinate (NaAOT) microemulsions (w = [H2O]/[surfactant] = 40) to reverse micelles of calcium dodecylbenzenesulfonate under conditions of high alkalinity (pH = 11) and a [Ca2+]:[CO32−] molar ratio of 1.4: 1. Mixing the microemulsions and reverse micelles gave a clear transparent solution, which slowly turned opaque after 3 h and produced a white macroscopic precipitate within 24 h.

Liu and coworkers (Liu et al. 2001) prepared nanosized CaCO3/SiO2 composite particles by the sol-gel process of CaCO3 and Na2SiO3 in an agitated tank reactor, with an average composite size of solgel coated CaCO3 of about 40 nm. CaCO3 nanoparticles have also been prepared using a microemulsion technique consisting of sodium dodecyl-sulphate (SDS)/isopentanol/cyclohexane/water, (Sunqing et al. 2000). Zhang et al. (Zhang et al. 2000) synthesized nanoparticles of calcium carbonate in the reaction system of Ca (OH)2/H2O-CO2. It was reported that the increase in temperature and mass fraction of the Ca (OH)2 suspension increased the particle size of the final product.
3.1.2 Motivation of the study

Miniemulsions technique for the preparation of calcium carbonate particles, are based on kinetic control during preparation; the particles are built from the center to the surface, and the particle structure is governed by kinetic factors (Landfester, K. 2001). Because of the dictate of kinetics, serious disadvantages such as lack of homogeneity and restrictions in the accessible composition have to be accepted. With the concept of "nanoreactors" where the essential ingredients for the formation of the nanoparticles on can take advantage of a potential thermodynamic control for the design of nanoparticles. This means that the droplets have to become the primary locus of the nucleation of the CaCO$_3$ precipitation. The CaCO$_3$ precipitation in such nanoreactors should take place in a highly parallel fashion, i.e. the synthesis is performed in $10^{18}$ - $10^{20}$ nanocompartments which are separated from each other by a continuous phase (Landfester, K. 2001). In miniemulsion calcium carbonate synthesis, the principle of small nanoreactors is realized as demonstrated in Figure 3.1.

![Miniemulsion principle](image)

**Figure 3.1. The miniemulsion principle.** For a typical oil-in-water miniemulsion, oil, a hydrophobic agent, an emulsifier, and water are homogenized by high shear to obtain homogeneous and monodisperse droplets in the size range of 30 to 500 nm. Source: Lanfester, K. *Adv. Mater.* 2001, 765.
In this chapter I described the synthesis of calcium carbonate nanoparticles by the inverse miniemulsion technique. It reveals that the morphology (shape) and CaCO₃ modification (polymorphs) can be tuned and stabilized effectively by various parameters. One of the advantages of using miniemulsion technique is that it can be designed to be highly selective depending on the purpose required. Some other advantages of using this technique is the low cost of operation since the organic phase can be reused, and has high separation rate due to the high surface area. In the present study reported that miniemulsion technique can be used to synthesize spherical amorphous calcium carbonate (ACC) particles. Together with the previous investigations on generation of inorganic nanoparticles in miniemulsion (Landfester, K. 2001, Antonietti et al. 2001), the results leads to a better understanding of the miniemulsion based calcium carbonate synthesis. These studies suggest that different approaches can be used for the generation of inorganic nanoparticles in order to obtain the required properties. The use of internal phase to control particle size and morphology has been a recent point of interest.

The optimal operating conditions for such systems vary from process to process. Factors such as ion concentration in phases, pH and temperature play a large role in the product properties. The emulsion concentration will be varied to produce different calcium carbonate particles. The effects of such variables will be the subject of future work. The main objective of this study is to develop a system to synthesize amorphous calcium carbonate (ACC) particles with controlled particles size in the range of nanometers, by the use of miniemulsion technique.
3.1.3 Miniemulsion

A system where small droplets with high stability in a continuous phase are created by using high shear (Blythe et al. 2000, Schork et al. 1999, Landfester et al. 2000) is classically called a “miniemulsion”. One of the tricks to obtaining stability of the droplets is the addition of an agent that dissolves in the dispersed phase, but is insoluble in the continuous phase. For a typical oil-in-water miniemulsion, an oil, a hydrophobic agent (or several), an emulsifier, and water are homogenized by high shear (see Figure 3.1) to obtain homogeneous and monodisperse droplets in the size range of 30 to 500 nm. The potential and the high impact of using miniemulsions for the synthesis of calcium carbonate will be presented.

In the present study inverse miniemulsions is prepared by emulsifying an aqueous inorganic salt solution in a continuous oil phase. The solution is dispersed in the continuous phase by means of an adequate amount of surfactant by fast stirring and the efficient use of ultrasonication. Here small droplets in the size range of 50-500 nm with sufficient stability are created. The method was modified to have two separate miniemulsions. This overcame the problem of an unstable external phase. For the preparation one inverse miniemulsion consisting of Ca$^{2+}$ ions containing droplets (Emulsion A) and another miniemulsion consisting of CO$_3^{2-}$ ions containing droplets (Emulsion B) are prepared. This preparation allowed the droplets of calcium and carbonate ions to interact within the system freely therefore having a more uniform concentration gradient in all droplets. Adding these two miniemulsions while applying high shear lead to permeation of ions from the emulsion B droplets into the emulsion A droplets across the continuous phase as see in Figure 3.2. This results in the precipitation
Miniemulsion-based Synthesis of Calcium Carbonate

of CaCO\textsubscript{3} nanoparticles. Precipitation of calcium carbonate in such miniemulsions, when carefully prepared, result in nanoparticles which have about the same size as the initial droplets, as could be shown by a combination of TEM and SEM. This means that the appropriate formulation of a miniemulsion suppresses coalescence of droplets or Ostwald ripening. The principle of miniemulsion based synthesis of calcium carbonate is realized as demonstrated in Figure 3.2.

Figure 3.2. Synthesis of calcium carbonate nanoparticles. (a) Preparation of two miniemulsions consisting of Ca\textsuperscript{2+} and CO\textsubscript{3}\textsuperscript{2-} ions containing droplets, (b) mixing two miniemulsions and then applying high shear, (c) formation of CaCO\textsubscript{3} nanoparticles.
3.1.4 Ostwald ripening and Collision process

Droplet growth can take place by two mechanisms: (a) Ostwald ripening and (b) collision process. The hydrophobic agent prevents Ostwald ripening (Ostwald, W. Z. 1901, Landfester et al. 2000). When a liquid emulsion is subjected to high shear, small droplets will result. There will still be a statistical distribution of droplet sizes. If emulsion is not stable enough the ions containing droplets diffuse from the smaller droplets into the larger ones. This results in a lower interfacial area (and interfacial energy), since the loss in interfacial area of the smaller droplets is larger than the gain in interfacial area of the larger ones. The reduction in interfacial energy is the driving force for degradation of the small droplets.

A hydrophobic agent functions to prevent Ostwald ripening by retarding droplets diffusion from the smaller droplets to the larger. Hydrophobic agent should be highly insoluble in the aqueous phase (so that they will not diffuse out of the droplets), and highly soluble in the continuous phase. Under these conditions, diffusion of the ions out of the smaller droplets results in an increase in the concentration of the hydrophobic agent in those particles (since, by definition hydrophobic agent are too insoluble in the aqueous phase to leave the droplet). The increase in free energy associated with the concentration of the hydrophobic agent balances the decrease from reduced interfacial area caused by Ostwald ripening, and, at some point, ripening stops.

It has been reported recently that (Landfester et al. 2002) the droplet coalescence caused by Brownian motion is still possible until the osmotic pressure and the Laplace pressure is equilibrated. This collision process can efficiently be reduced by the use of a suitable
surfactant (this maintain particles separation by steric hindrance or electrostatic stability). The stability of inorganic particles by like-charge repulsion in aqueous media can be described by the well-known DLVO theory (Hunter, R. J. 1987, Ross et al. 1988). This theory considers the two potentials, repulsive and attractive, which develop from the electrostatic (Parkanyi–Berka et al. 1988) and London forces (Parfitt et al. 1966, Novotny, V. 1978) that can exist between two particles. A third potential, known as the steric potential, arises from the adsorption of the head group of a surfactant onto the surface of a particle while the uncharged tail stays in the solution providing steric stabilization (Napper, D. H. 1983 & 1977, Barrett, K. E. 1975, Parfitt et al. 1966). The sum of these three potentials determines whether two particles will flocculate or remain dispersed in the medium. Steric potential is the primary means of stabilization in nonaqueous media.

Figure 3.3. Suppression of Ostwald ripening. The hydrophobe suppresses Ostwald ripening. The driving force is to avoid an off-balance of the osmotic pressure. Source: Landfester, K. Macromol. Symp. 150, 171-178 (2000).

In Figure 3.3 the effect of the osmotic pressure is shown schematically. In the case of ions diffusion from the smaller droplet into the larger droplet (the usual Ostwald ripening) the pressure in the smaller droplet would increase more that the Laplace pressure driven by the droplet interfacial tension, and the process is therefore inhibited.
Miniemulsion-based Synthesis of Calcium Carbonate

(a) Reaction product of Succinic anhydride
Polybutene-succinimide pentamine (MW = 950-2500 g/mol)

(b) Poly [(ethylene-co-butylene)-b-(ethylene oxide)]
Poly (ethylene-co-butylene) MW = 3700 g/mol
Poly (ethylene oxide) MW = 3600 g/mol

(c) N-Acetyl-L-Glutamic acid (MW = 189.17g/mol)

Figure 3.4. Chemical structure of surfactants. Chemical structure of (a) & (b) surfactant and (c) additive used in the precipitation of calcium carbonate. Source: (a) Lubrizol, (b) Schlaad et al. Macromolecules, 2001, 34, 4302, (c) Merck.
3.1.5 Transport Mechanism

The transport of calcium ions through continuous phase (oil phase) is an integral part of the ‘facilitated transport’ to form precipitated calcium carbonate. Polybutene-succinimide pentamine and poly [(ethylene-co-butylene)-b-(ethylene oxide)] were used as surfactants in the present study. The molecular structure of these surfactants can be seen in Figure 3.4. The rate of transport has been found to be dependent on the concentration gradient across the continuous phase, temperature and pH (Moreno et al. 1999). It has been seen from similar studies that a lower pH and lower concentration in the internal phase promotes ion transfer across the continuous phase.

At stage I, surfactant (HN) forms an oil soluble complex with Ca\(^{2+}\) ions as shown in the equation below.

\[
Ca^{2+} + 2HN \rightarrow CaN_2 + 2H^+
\]

Due to its high solubility in oil, this complex then penetrates through the continuous phase much more easily from emulsion B droplets to emulsion A droplets. Here it reacts with the \(CO_3^{2-}\) to form \(CaCO_3\) particles with the release of the surfactant (HN) in the equation below:

\[
CaN_2 + CO_3^{2-} + H_2O \rightarrow CaCO_3 \downarrow + 2HN + 2OH^-
\]

The remaining surfactant picks up a H\(^+\) from the aqueous phase to form a neutral surfactant, then diffuses back through the continuous phase to the external surface and reacts with Ca\(^{2+}\)to form more complexes. This continues until all the calcium ions have been reacted with the \(CO_3^{2-}\) and the reaction can not go any further. It should be noted that sodium salts do not tend to form a complex with the surfactant as they are insoluble in the organic phase. The
absorption of H\(^+\) is more stable and therefore more likely to occur. Figure 3.5 shows the overall mechanism involved.

**Figure 3.5. Transport Mechanism.** Facilitated Transport Mechanism of surfactant to forms an oil soluble complex with Ca\(^{2+}\) ions containing droplets, due to its high solubility in oil, this complex then penetrates through the continuous phase much more easily from emulsion B droplets to emulsion A droplets. Here it reacts with the CO\(_3^{2-}\) to form CaCO\(_3\) particles with the release of the surfactant (HN). The remaining surfactant picks up a H\(^+\) from the aqueous phase to form a neutral surfactant, then diffuses back through the continuous phase to the external surface and reacts with Ca\(^{2+}\) to form more complexes. This continues until all the calcium ions have been reacted with the CO\(_3^{2-}\) and the reaction can not go any further.
3.1.6 Surfactant Selection

Hydrophile – Lipophile Balance also known as HLB was a method proposed by Griffin, as a guide to select an optimal emulsifying agent (Garciaa et al. 1989). It has been shown by Sherman that HLB depends on both concentration as well as phase volumes of the oil and water.

HLB is used to characterize most oil phases using solubility parameter (SP) values. The higher the HLB the higher the solubility in water. The SP values may also be derived from basic physical properties (Vaughan et al. 1990). The HLB value is derived from the following equation:

$$HLB = 4\left(\frac{SP + 7}{8}\right)$$

This equation is used to predict the stability of emulsions and select optimal emulsifying agents. Therefore, generally, a lower HLB number is used for water in oil (W/O) emulsifications and a higher HLB number is used for oil in water (O/W) emulsifications.

3.2 Experimental Section

3.2.1 Materials. All chemicals were of analytical grade and used without further purification.

3.2.2 Preparation of Calcium carbonate. Calcium carbonate was synthesized in reverse micelles at a \([\text{Ca}^{2+}]: [\text{CO}_3^{2-}]\) molar ratio of 1:1 and pH value of 8.2. In a typical synthesis, one miniemulsion is prepared by using Lubrizol (polybutene-succinimide pentamine) or poly [(ethylene-co-butylene)-b-(ethylene oxide)] (Figure 3.4) as surfactants. 750 mg of surfactants were dissolved in 9 g of cyclohexane.
To this solution 10 mL of aqueous calcium chloride (2 M, pH 11) was added, and the mixture stirred using magnetic stirrer at its maximum speed, and this homogenized solution was sonicated in ice bath for 2 min (amplitude 70%, BRANSON Digital Sonifer Model-450-D, Microtip: diameter 6.5 mm) to produce a milky white emulsion.

A similar procedure was repeated to prepare the CO$_3^{2-}$ ion containing miniemulsion. Here 10 mL of aqueous sodium carbonate (2 M, pH 12) were used as dispersed phase. Adding these two miniemulsions (Ca$^{2+}$ and CO$_3^{2-}$ ion containing droplets) while applying high shear leads to the calcium carbonate nanoparticles as shown in Figure 3.2. To study the role of additives in the calcium carbonate crystallization, 20 mg N-acetyl-L-glutamic acid (monomers), were dissolved both in CaCl$_2$ and Na$_2$CO$_3$ solution. I also tested the effect of magnesium ions on CaCO$_3$ precipitation by adding 2 ml of 100 mM magnesium chloride to the polymer surfactant solution mix.

SEM studies on the CaCO$_3$ crystals were carried out using a Hitachi S-5200 field-emission scanning electron microscope at acceleration voltages of 10 or 4 kV after rotary coating with 3nm of platinum at an angle of 45$^\circ$ in a Balzers BAF 300 (Kindly thanks Prof. Dr. Paul Walther and PD Dr. Ziegler for SEM analysis). Powder X-ray diffraction studies on the crystal powder were carried out using a Siemens D500 X-ray diffractometer with Cu K$\alpha$ radiation at 40 kV and 30 mA (Kindly thanks Dr. Bernd Heise for XRD analysis). Raman spectra were recorded using a CRM 200 confocal Raman spectrometer, WiTec, Ulm, Germany. The exciting source was a SGL-2200 laser operating at 532 nm with a power of about 200 mW. The scattered light was collected at an angle of 180$^\circ$ (back-scattering). Typical spectral resolution was 3 cm$^{-1}$. To avoid the transformation of less stable
polymorphs into calcite the laser beam is scanned in a field of 200 x 200 µm². The total exposure time was 20 sec. (Courtesy: Dr. Hild & Dr. Ute Schmidt for Raman analysis).

For TEM, a drop of the diluted samples air dried onto carbon-coated Formvar films on copper grids, followed by washing the grids in cyclohexane and air drying. TEM was performed in bright field mode using a Zeiss EM 10 electron microscope operating at 80 kV. EDX analysis was used to confirm the presence of carbon, oxygen, calcium and magnesium elements. For FTIR spectroscopy, samples were washed with cyclohexane and air dried and then analyzed using KBr disks (Courtesy: Elvira Kaltenecker for FTIR analysis). Samples for TGA were prepared by washing and air drying the emulsion, and then heated from 25 to 800 °C at a rate of 5 °C/min using a Mettler Toledo TGA/SDTA 851 (Courtesy: Marlies Fritz for TGA analysis).

3.3 Results and Discussion

3.3.1 Synthesis of Amorphous Calcium Carbonate.

Miniemulsion comprising aqueous calcium chloride, sodium carbonate, Lubrizol (polybutene-succinimide pentamine) (Figure 3.4a), and cyclohexane was prepared at a [Ca²⁺]: [CO₃²⁻] molar ratio of 1:1 and pH value of 8.2, and sonicated in ice bath for 2 min (detailed procedure for the preparation of miniemulsions as mentioned before in section 3.2.2) to produce a milky white emulsion. When left unstirred at room temperature for 8 hours, the final miniemulsions (A+B) separated into upper and lower layers consisting of a transparent liquid phase (cyclohexane) and milky white precipitate phase (aqueous phase with CaCO₃ particles), respectively (see in Figure 3.6). The obtained samples before and after washing will be analyzed.
Electron microscope images of amorphous calcium carbonate (ACC) generally show that ACC is composed of spheres (Becker et al. 2003, Brećević et al. 1989). ACC prefers a spherical shape in solution to minimize its contact area with solution (Raz et al. 2003). TEM images of samples extracted from the lower layer after 2 days revealed electron dense spherical particles that were variable in size range between 10-60 nm (Figure 3.7) and SEM images of the dried and washed samples revealed aggregates of spherical particles is in the size range between 80-100 nm (Figure 3.8).

Figure 3.6. Phase separation. Phase Separation of the miniemulsions A+B after 8 hours.

Figure 3.7. TEM image of an ACC. TEM image of an amorphous calcium carbonate (ACC) prepared in the presence of polybutene-succinimide pentamine by miniemulsion technique.
Figure 3.8. SEM microphotographs of ACC. SEM microphotographs of (A) amorphous calcium carbonate (ACC) obtained in the presence of polybutene-succinimide pentamine by miniemulsion technique, (B) higher-magnification showing detailed texture.

Since the experimental solutions contain NaCl, we performed EDS to analyze the chemical composition of the crystals. The results verified that the spherical aggregates are CaCO$_3$ with only a little Na and Cl present.
Selected area X-ray diffraction (Figure 3.9), as well as FTIR (Figure 3.10 & 3.11), and Raman spectroscopy (Figure 3.12 upper and lower) obtained from emulsion before washing [in emulsion and emulsion in dried state (after evaporating cyclohexane)] samples, indicated that the spheres were amorphous calcium carbonate (CaCO3:H2O and CaCO3:6H2O respectively, see in Figure 3.12) [more detailed studies on phase analysis by IR and Raman spectroscopy, see section 3.3.1.1]. XDR pattern recorded for the emulsion dried (after evaporating cyclohexane) samples shows (Figure 3.9a) only sodium chloride (NaCl) signals and does not show any crystalline peak. These NaCl signals are arise from the biproduct of the reaction.

![XRD patterns of CaCO3](image)

**Figure 3.9. XRD patterns of CaCO3.** XRD patterns of CaCO3 particles obtained in the presence of polybutene-succinimide pentamine (a) emulsion dried and (b) emulsion washed. [More detailed studies on phase analysis see section 3.3.1.1]
### FTIR Peak Table

<table>
<thead>
<tr>
<th>Modifications</th>
<th>Reference peak cm⁻¹</th>
<th>FTIR peaks from experimental data cm⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>In emulsion</td>
</tr>
<tr>
<td>ACC¹ CaCO₃.H₂O</td>
<td>3319 / 3232, 2553, 2472, 2270, 1641, 1421, 1409 / 1069, 873 / 700</td>
<td>3314&quot; / 3220&quot;</td>
</tr>
<tr>
<td></td>
<td>765, 674</td>
<td>673</td>
</tr>
<tr>
<td>ACC² CaCO₃.6H₂O</td>
<td>3467 / 2515, 1641 / 1421, 1082, 877, 814 / 571</td>
<td>3465 / 2516</td>
</tr>
<tr>
<td>Vaterite³</td>
<td>2507, 2356, 1489, 1432, 1408 / 1089, 877 / 745, 668</td>
<td>2506, 1484&quot;, 1438&quot;</td>
</tr>
<tr>
<td>Calcite ⁴</td>
<td>713</td>
<td>713</td>
</tr>
</tbody>
</table>

¹large shift due to the presence of solvent (cyclohexane) / surfactant (polymer) or could be overlapping with other peaks.

**Figure 3.10. FTIR Peak Table.** FTIR analysis¹⁹ shows summary of the experimental results of the nucleation of CaCO₃ obtained in the presence of polybutene-succinimide pentamine (a) in emulsion, (b) emulsion dried, (c) emulsion washed (see Figure 3.11, for the corresponding spectra). [More detailed studies on phase analysis see section 3.3.1.1]

---

¹⁹¹Catherine, et al. (1977); ²Kennedy, et al. (1987); ³Weir, et al. (1961); ⁴White, et al. (1974)
Figure 3.11. Summary of the experimental results. IR spectra show the presence of ACC obtained in the presence of polybutene-succinimide pentamine [see Figure 3.10, for the corresponding IR peak table]: (a) in emulsion (CaCO$_3$:H$_2$O), (b) emulsion dried (CaCO$_3$·6H$_2$O), (c) emulsion washed (CaCO$_3$: vaterite phase). [More detailed studies on phase analysis see section 3.3.1.1]
Figure 3.12. Raman analysis show the presence of ACC. Raman analysis show: upper) Raman peak table shows summary of the experimental results: (a) in emulsion (CaCO$_3$:H$_2$O), (b) emulsion dried (CaCO$_3$:6H$_2$O), (c) emulsion washed (CaCO$_3$: vaterite phase) and lower) Raman spectra for the corresponding analysis [More detailed studies on phase analysis see section 3.3.1.1].
The initial composition of ACC in solution has not been determined due to the lack of available characterization techniques (Rieger et al. 2000). However, Gower and coworkers (Gower et al. 2000, Olszta et al. 2003) has found that a polymer-induced liquid-precursor process occurs and suggested that polymer induces the liquid-liquid phase separation of droplets of a mineral precursor. A recent paper postulated liquid-liquid phase segregation, and ACC particles are formed from a loss of water of the highly concentrated solution by the gelation process (Faatz et al. 2004). It can be speculated that ACC prefers a spherical droplet shape and contains a lot of water in solution.

Our results can be rationalized by considering ACC as liquid-like colloids, which contain many water molecules. When excess calcium chloride is mixed with sodium carbonate, the zeta potential of CaCO₃ precipitates is positive because calcium cations are adsorbed on the surface of precipitates (Chibowski et al. 2003). Even for equimolar solutions, the zeta potential of CaCO₃ is still small positive during first 30 min (Chibowski et al. 2003). Here a method of slow diffusion of carbon dioxide (CO₂) into CaCl₂ solution is used, and the excess calcium ions exist in CaCl₂ solution. On one hand, ACC, which spontaneously formed first in CaCl₂ solution, preferentially adsorbs calcium ions to form stable positively charged ACC colloids with lots of water. On the other hand, ACC colloids have a tendency to aggregate, coalesce, dehydrate, and solidify.

Usually, the coexistence of calcite, vaterite, and amorphous calcium carbonate (ACC) polymorphs is an indication of a metastable state (Colfen et al. 2001), and the ACC to vaterite polymorphs will be changed into calcite on extended aging time through a solvent mediated process (Spanos et al. 1998, Nassrallah-Aboukais et al. 1998,
Lopezmacipe et al. (1996) in solution. However, even though our samples were aged in solution and also dried state (after evaporation of cyclohexane) for long times (e.g. 14 days); the XRD, FT/IR, Raman and SEM analyses exhibited the same ACC phase structure and morphology [more detailed studies on phase analysis by IR and Raman spectroscopy, see section 3.3.1.1]. This indicates that the ACC polymorphs can be stabilized under this experimental condition.

Another reason for the stabilization of ACC, what was expected that a block copolymer-type surfactant would provide the required stabilization of the inorganic particles in an oil phase by anchoring one block to the surface of the inorganic particles, while the other block remained soluble in the surrounding media, thus providing steric stabilization against approaching particles (Erdem et al. 2000). This kind of interaction is supposed to be in the form of a weak physical adsorption. When an AB-type copolymer is used (e.g., polybutene-succinimide pentamine, which we used in the present study), a brush-type orientation is expected. For example, with polybutene-succinimide pentamine surfactant, the poly (butene) block was expected to interact with the particle surface with the succinimide pentamine block extending into the medium. This succinimide pentamine block would be compatible with the inorganic particles as the dispersion medium, thus providing steric stabilization (Erdem et al. 2000).

In contrast, analogous studies on washed samples extracted from lower layer showed the presence of vaterite as well as small amount of calcite modifications. The XRD pattern of the washed samples is shown in Figure 3.9b and clearly shows transformation of amorphous calcium carbonate (ACC) to vaterite and calcite
modifications. This is also supported by FTIR and Raman measurements of the washed samples which show characteristic vaterite & calcite bands (Figure 3.11c, 3.10 for the corresponding IR peak table for washed sample and Figure 3.12a, b respectively). This indicates that the spherical particles consist predominantly of vaterite and partly calcite [more detailed studies on phase analysis and phase transformation by IR and Raman spectroscopy, see section 3.3.1.1].

Figure 3.13. TGA profile and FTIR spectra recorded from CaCO₃ particles. Upper) TGA profile & lower) FTIR spectra recorded from CaCO₃ particles obtained in the presence of polybutene-succinimide pentamine (washed sample).
TGA profiles of the washed calcium carbonate particles indicated that surfactant molecules were intimately associated with the spherical particles (Figure 3.13 upper). A weight loss of 30% was detected between 200 and 300 °C with attributed primarily to the decomposition of surfactant molecules. This was consistent with FTIR spectra of washed and dried samples (Figure 3.13 lower) which showed methylene symmetric and antisymmetric vibration (2926, 2954 cm⁻¹), –NH₂ and >NH (3452 cm⁻¹), CH₃ (1440 cm⁻¹), >CH₂ (2860 cm⁻¹) bands associated with surfactant molecules. This clearly shows that surfactant chemically adsorbs on the CaCO₃ surface, which interact with the amine end group of polybutene-succinimide pentamine is basic and accepts protons or donates an electron pair, which results in a concentration-dependent negative zeta potential on the surface of hydrophilic CaCO₃ particles in low dielectric media such as cyclohexane. This means that the amine end group on this polymeric surfactant can readily interact with reactive hydroxyl groups present on the surface of the CaCO₃ particles. Our assumption and the possible reactions were given in Eq.1-4.

\[
\begin{align*}
\text{CaCO}_3 & \leftrightarrow \text{Ca}^{2+} + \text{CO}_3^{2-} \quad (1) \\
\text{CO}_3^{2-} + \text{H}_2\text{O} & \leftrightarrow \text{HCO}_3^- + \text{OH}^- \quad (2) \\
\text{H-N} & \leftrightarrow \text{H}^+ + \text{N}^- \quad (3) \\
\text{N}^- + \text{H}_2\text{O} & \leftrightarrow \text{HN}^+ \text{OH}^- \quad (4)
\end{align*}
\]

The surfactant (H-N) adsorption can be explained by the change of solubility of CaCO₃ and surfactant in aqueous solution. When the pH of the solution increases the solubility of calcium carbonate in solution decreases (Eq.1 and 2) and the solubility of surfactant increases (Eq.4). In pH range 9 to 11 surfactant adsorption can be
expected on the surface of the calcium carbonate and at this stage vaterite polymorph is stabilized. But at pH 12 the solubility of calcium carbonates become very low and the concentration of Ca-ion in the solution become very low. Therefore, in this pH range only slow adsorption can be expected and at this stage vaterite is transformed to more stable calcite polymorph. This might be the reason for the spherical particles consist predominantly of vaterite and partly calcite.

According to atomic surface structure modeling, the (001), (101), and (110) planes are positively charged and contain exposed Ca ions; thus, negatively charged polymer groups can adsorb on the plane (Yu et al. 2002). It is understandable given that higher surfactant concentration (namely, the increase of concentration of reactive functional groups) could translate into better CaCO$_3$ binding efficiency and adsorption ability. Thus, preferential adsorption of the polymer surfactant on vaterite relative to calcite that can at least balance the relative loss in the crystallization energy (Colfen et al. 2001) could results in the crystallization of vaterite. Though these planes would block for further growth, the stabilization of the vaterite surface by the reactive functional groups of the polymer surfactant in aqueous solution may prevent phase transformation of the metasatable polymorphs owing to the interaction between Ca$^{2+}$ and the carbonyl or amine groups of the polymer surfactant.

From the above results we can speculate that the polymer surfactant could form complex micelles for the assembly of ACC spherical aggregates. It is difficult to fully understand the mechanism implication of the effect of the polymers surfactant over the crystallization of CaCO$_3$ and this will be the subject of future work.
3.3.1.1 Transformation from calcium carbonate monohydrate via hexahydrate to vaterite modifications: Characterization by FT/IR and Raman spectroscopy

Numerous investigations have been performed on the equilibrium of the CaCO$_3$–CO$_2$–H$_2$O calcocarbonic system and on the kinetics of calcium carbonate precipitation from these solutions. A large domain of supersaturation exists where CaCO$_3$ precipitation is not observed, although predicted by thermodynamics (Tlili et al. 2001). Either in the laboratory (Legrand et al. 1981, Roques et al. 1964, Dedek, J. 1966) or in the natural environment, (Abu-Sharar, T. M. 1990, Amrhein et al. 1993) supersaturated solutions can be kept in this metastable state, without any appearance of CaCO$_3$ crystals in one of the three possible crystal forms of anhydrous calcium carbonate, namely calcite, aragonite or vaterite. Within the metastable domain, three other forms of calcium carbonate have been mentioned in the literature. All these varieties are hydrated: amorphous calcium carbonate, calcium carbonate hexahydrate, CaCO$_3$:6H$_2$O and calcium carbonate monohydrate, CaCO$_3$:H$_2$O. Various authors have suggested these hydrated forms to be the precursors of the formation of the anhydrous forms of CaCO$_3$. The nucleation of an anhydrous form would occur when the solubility product of one of these hydrated forms is exceeded. The knowledge of the formation conditions and the properties of these hydrates are important to obtain a better understanding of the fundamental processes of their transformation into stable forms.

Amorphous calcium carbonate (ACC) is unstable in humid air, in which it transforms into anhydrous crystal phases. FT/IR and Raman spectroscopy was used to investigate the phase transformation. Amorphous calcium carbonate (ACC) in the solution (miniemulsion
Miniemulsion-based Synthesis of Calcium Carbonate

consist of cyclohexane and aqueous phase with CaCO₃:H₂O particles), in dried condition (sample consist of CaCO₃:6H₂O particles after evaporating cyclohexane), and also after washing the sample by using distilled water for several times (only consists CaCO₃ (vaterite) and adsorbed surfactant) will be analyzed. The phase analysis of the above mentioned sample preparations; indicate the transformation from calcium carbonate monohydrate via hexahydrate to vaterite modifications. The FT/IR spectra (Figure 3.11) of calcium carbonate monohydrate (CaCO₃:H₂O), calcium carbonate hexahydrate (CaCO₃:6H₂O) and vaterite were in good agreement with previous reports (Catherine et al. 1977, Kennedy et al. 1987, and Weir et al. 1961) and are shown in Figure 3.10. Transformation of ACC into a crystalline form involves the release of water molecules included in ACC (Aizenberg et al. 2003).

(a) FTIR analysis of ACC in miniemulsion

The infrared spectra of calcium carbonate (CaCO₃) phase in miniemulsion we studied and the spectra for stable calcium carbonate monohydrate (CaCO₃:H₂O) studied by Addadi et al. and Catherine et al. (Addadi et al. 2003, Catherine et al. 1977) are basically the same (Figure 3.11a). Stable calcium carbonate monohydrate (CaCO₃:H₂O) has an absorption peak at 873 cm⁻¹ (Catherine et al. 1977), which is broader than the equivalent peak of the crystalline polymorphs (Figure 3.11a). CaCO₃:H₂O is characterized by a split peak at the asymmetric stretch of the carbonate ion at 1410 and 1070 cm⁻¹ (Farmer, V.C 1974). This is an indicative of a lack of symmetry in the environment of the carbonate ions. Stable CaCO₃:H₂O further more has a very broad and weak absorption at around 700 cm⁻¹, in contrast to calcite have sharp peaks at 713 cm⁻¹. Thus the absence of a peak at 713 cm⁻¹ indicates that well
ordered calcite is not present, but only calcium carbonate monohydrate. The presence of CaCO₃:H₂O is again confirmed by Raman analysis (Figure 3.12d), which shows broad peak at around 1061 cm⁻¹, and usually a broad featureless hump around a 154-390 cm⁻¹. This is in contrast to the crystalline forms of calcium carbonate that all have a series of relatively sharp peaks (Addadi et al. 2003). Further more CaCO₃:H₂O has a weak absorption band at around 725 and 872 cm⁻¹, in contrast to calcite have sharp peaks at 711 cm⁻¹ (Clarkson et al. 1992, Tlili, et al. 2001, Gabrielli et al. 2003).

(b) FTIR analysis of ACC in dried condition (after evaporating cyclohexane)

The infrared spectra of calcium carbonate (CaCO₃) phase in dried sample (after evaporating cyclohexane), shows the sharp peak at 877 cm⁻¹ and broad and weak absorption at around 614/571 cm⁻¹ (Figure 3.11b), which is characteristic peaks for calcium carbonate hexahydrate [CaCO₃:6H₂O] (Kennedy et al. 1987). The result shows that the transformation from CaCO₃:H₂O to CaCO₃:6H₂O occurred after the removal of cyclohexane from the sample. This indicates that the calcium carbonate monohydrate in the miniemulsion, the approach of water molecules due to humid air is inhibited by the oil phase (cyclohexane). After the evaporation of cyclohexane from the sample, CaCO₃:H₂O transform to CaCO₃:6H₂O by taking water molecules from humid air. The Raman spectra of this sample were showed similar to CaCO₃:H₂O spectra. Figure 3.12c shows Raman spectra of stable ACC (CaCO₃:6H₂O) are characterized by a broad peak in the lattice frequency region 154-390 cm⁻¹, that occasionally appears as a continuous baseline rise rather than a discrete peak (Addadi et al. 2003). The carbonate symmetric stretching peak at approximately 1067 cm⁻¹ of
stable ACC (CaCO$_3$:6H$_2$O) is also substantially broadened, relative to the crystalline counterparts. Absorption band at around 722 and 1418 cm$^{-1}$, which again confirmed the presence of CaCO$_3$:6H$_2$O (Clarkson et al. 1992, Tlili, et al. 2001).

(c) FTIR analysis of ACC in washed and air dried condition

The infrared spectra of calcium carbonate (CaCO$_3$) phase in washed and air dried sample, shows the sharp peak at 877 / 745 cm$^{-1}$ (Figure 3.11c) and broad and weak absorption at around 668 cm$^{-1}$ which is characteristic peaks for vaterite (Weir et al. 1961). Raman analysis support this findings (see Figure 3.12a, b). It is well known that less stable form (e.g., amorphous calcium carbonate) transforms readily and irreversibly into metastable vaterite via to the most stable calcite form through a solvent-mediated process (Spanos et al. 1998). A similar mechanism could happen while washing and drying the samples. We can conclude that the resulted unstable vaterite phase during washing could be transiently stabilized by the adsorbed polymer surfactant and the some of the vaterite phases which are not occluded by the polymer surfactant, could be precipitate and subsequently transforms into the most stable calcite phase.

Ostwald-ripening and solvent-mediated recrystallization process depends on the presence of a favorable difference in interface energy as a driving force (Cölfen et al. 2001). It must be underlined that calcium carbonate monohydrate (CaCO$_3$:H$_2$O) and calcium carbonate hexahydrate (CaCO$_3$:6H$_2$O) presented before were stable against aging, that is, there is no positive interface energy difference which drives the
process. This means that at coverages above $S_{cr}^{20}$, the system does not loose (or even gains) energy by exposing crystal surface that can adsorb polymer. It is a question of thermodynamics (Cölfen et al. 2001) that at the $S_{cr}^{4}$ the interface energy is zero, while it is by definition negative at higher concentrations (all these considerations just hold for crystals being large compared to the polymer).

This goes well with the observation that stable vaterite particles (although vaterite is metastable in pure water) can be produced in the presence of organic additives that can stabilize the vaterite surface, such as polybutene-succinimide pentamine. It is assumed that in this case the preferential adsorption of the polymer surfactant on vaterite relative to calcite can at least balance the relative loss in the crystallization energy.

3.3.2 Synthesis of CaCO$_3$ using poly [(ethylene-co-butylene)-b-(ethylene oxide)] and also in combination with magnesium chloride and $N$-acetyl-$L$-glutamic acid

Studies were undertaken to investigate the influence of poly [(ethylene-co-butylene)-b-(ethylene oxide)] (Figure 3.4b), surfactant in combination with low molecular weight additives like magnesium chloride and $N$-acetyl-$L$-glutamic acid (Figure 3.4c).

---

20 According to Cölfen and coworkers (Cölfen et al. 2001), adsorption process, can define an equilibrium constant $k = \frac{[\text{polymer}_{\text{ads}}]}{[\text{polymer}]}$ per unit area of CaCO$_3$ and, evidently, there must be a critical surface coverage $S_{cr}$ by the polymer to promote morphological changes if the growing crystal.
3.3.2.1 CaCO$_3$ precipitation using poly [(ethylene-co-butylene)-b-(ethylene oxide)]

SEM images of CaCO$_3$ samples obtained from reaction mixtures comprising aqueous calcium chloride, sodium carbonate, poly [(ethylene-co-butylene)-b-(ethylene oxide)] and cyclohexane was prepared at a [Ca$^{2+}$]: [CO$_3^{2-}$] molar ratio of 1:1 and pH value of 8.2, showed the presence of three different morphologies such as needle-like nanofilaments, spherical aggregates and also some irregular crystals (Figure 3.14). The obtained CaCO$_3$ particles were isolated, washed and air dried for characterization.

Figure 3.14. SEM microphotographs of CaCO$_3$. SEM microphotographs of CaCO$_3$ obtained in the presence of poly [(ethylene-co-butylene)-b-(ethylene oxide)] by miniemulsion technique.

The presence of calcite, aragonite and amorphous calcium carbonate phases was confirmed by IR due to the observation of bands at 877 cm$^{-1}$ and 715 cm$^{-1}$ for calcite, 1476, 1115, 1034, 1074, & 907 cm$^{-1}$ for aragonite and 1636, 804, and 693 cm$^{-1}$ for amorphous calcium carbonate (Figure 3.15 upper). The slight discrepancy between
experimental and published IR values can be attributed to the required grinding of a CaCO₃ sample with KBr to produce pellets.

![FTIR spectra & TGA recorded from CaCO₃](image)

Figure 3.15. FTIR spectra & TGA recorded from CaCO₃. Upper) FTIR spectra recorded from CaCO₃ particles obtained in the presence of poly [(ethylene-co-butylene)-b-(ethylene oxide)] & lower) TGA profile showing a weight loss of surfactant between 180 – 285 °C.
This results in a decrease in particle size which causes certain bands to shift to higher wavenumbers, and change in intensity (the Christiansen effect [Rao et al. 1963]). The sample was principally aragonite, containing 20% of calcite, as detected by XRD (data not shown) and 15% of ACC detected by IR. IR was particularly valuable for detecting amorphous calcium carbonate phases of CaCO₃; XRD was more sensitive for the identification of crystalline phases. TGA (Figure 3.15 lower) profile shows ~10% of polymer surfactant is occluded in the samples. The experiment demonstrated that amorphous calcium carbonate (ACC) was the first phase precipitated. ACC is the most soluble, and therefore the least stable form of calcium carbonate and it is the first phase precipitated from solution if the supersaturation exceeds the solubility product of ACC. The stability of this phase depended on the polymer surfactant concentration in solution, and increased dramatically with increasing surfactant concentration (Meldrum et al. 2001). Poly[(ethylene-co-butylene)-b-(ethylene oxide)] can considerably affect crystal nucleation, often stimulating aggregation. Small concentrations of organic additives typically affect crystallization via adsorption of the active molecules at a number of growth sites on the surface of pre-critical nuclei, preventing growth beyond the pre-critical size (Meldrum et al. 2001). Incorporation of small amount of poly[(ethylene-co-butylene)-b-(ethylene oxide)] into a crystallite may create internal strain, thus increasing solubility and the critical size required for a stable precipitate. This could be one of the reasons for the transformation of ACC to more stable aragonite and calcite phases.
3.3.2.2 CaCO₃ precipitation using [(ethylene-co-butylene)-b-(ethylene oxide)] in combination with magnesium chloride

(a) Magnesium chloride in one inverse miniemulsion

Magnesium exerts a significant influence on calcium carbonate precipitation and can either be incorporated within the calcite lattice when Mg:Ca ratio in solution is low, or induce aragonite precipitation when magnesium concentration is sufficiently high (Loste et al. 2003). The Mg²⁺ ion is more strongly hydrated than Ca²⁺ and is strongly adsorbed onto the surface of growing calcite crystals. Dehydration of the Mg²⁺ ion prior to incorporation in the calcite lattice creates a barrier to the growth of calcite nuclei. Magnesium calcite is more soluble than pure calcite, which renders this phase energetically less stable (Loste et al. 2003). In biological systems, both Mg and specialized organic macromolecules may be used to stabilize less stable mineral phase [e.g., ACC] (Aizenberg et al. 2002). Because of this, experiment was carried out using poly [(ethylene-co-butylene)-b-(ethylene oxide)] in combination with magnesium chloride as a second additive. The work described here investigates the formation of magnesium calcites with the aim of studying the role of magnesium in controlling calcite morphology by stabilization of less stable CaCO₃ phases (e.g., vaterite and aragonite).

When magnesium chloride is used (by adding 2 ml of 100 mM magnesium chloride to the polymer surfactant solution mix) in one inverse miniemulsion consisting of Ca²⁺ ions containing droplets, the final emulsions (A+B) separated into two layers, including a clear liquid upper phase and white macroscopic precipitate as lower phase. The obtained CaCO₃ particles were isolated, washed and air dried for characterization. SEM images taken from lower precipitated materials
Miniemulsion-based Synthesis of Calcium Carbonate

consisted of extended plate-like crystals with some stacked arrays of spherical aggregates upon these crystal plates (Figure 3.16).

![SEM microphotographs of CaCO$_3$. SEM microphotographs of CaCO$_3$ obtained in the presence of poly [(ethylene-co-butylene)-b-(ethylene oxide)] in combination with magnesium chloride.](image)

The plates varied in size from 130 to 200 nm, and these extended plate-like crystals consisting of stacked spherical aggregates, suggesting that the superstructures developed within the miniemulsion liquid phase and not by uni-directional outgrowth from the walls of the reaction vessel. Moreover, the progressive change in the size of the plates indicated that the plates continued to grow principally in width rather than thickness. Although the individual plates were often disordered and showed extensive stacked spherical aggregates possibly due to washing the samples from reaction mixtures. They were structurally intact, suggesting the presence of strong interparticle contacts between adjacent lamellae. Significantly, high magnification SEM images showed close morphological alignment of the extended plate’s pattern of growth (Figure 3.16). FTIR
Miniemulsion-based Synthesis of Calcium Carbonate

(Figure 3.17) analysis shows the presence of both vaterite and aragonite modifications, but latter is the predominant form.

The stability of these phases (vaterite and aragonite) depended on the Mg:Ca ratio in solution, and increased dramatically with increasing Mg (Loste et al. 2003). The experiments demonstrate that Mg incorporation within vaterite and aragonite phases significantly retards transformation into calcite phase, and that this effect increases with the quantity of Mg occluded within the vaterite and aragonite structure. EDX analysis confirmed the presence of magnesium in the sample.

The formation of extended plate-like crystals with some stacked arrays of spherical aggregates upon these crystal plates can be attributed to the initial production of ACC particles (it appeared as spherical particles) which aggregates to form a extended plate-like crystals. Transformation into more stable polymorphs (vaterite and aragonite) occurs by a continuous dissolution / re-precipitation.
mechanism (Sawada, K. 1997), which occurs to maintain the overall morphology.

(b) Magnesium chloride in both inverse miniemulsions

When magnesium chloride is used in both (by adding 2 ml of 100 mM magnesium chloride to the polymer surfactant solution mix in both miniemulsions) the inverse miniemulsion consisting of Ca\(^{2+}\) and CO\(_{3}^{2-}\) ions containing droplets, it resulted in the nucleation of spherical crystals in the size range between 100 - 500 nm (Figure 3.18) and also found several octahedral single NaCl crystals in the size range between 400-500 nm (Figure 3.18). The product from solution was isolated as soon as possible, and was identified as amorphous calcium carbonate (ACC) morphologically (it appeared as spherical particles).

EDX analysis on these spherical crystals (Figure 3.18) showed peaks for calcium, carbon, oxygen, magnesium and octahedral crystals shows sodium and chlorine indicating that the spherical particles are contaminated with NaCl. Why this octahedral single NaCl crystal occurs only when magnesium chloride is used in both miniemulsions is unclear at the present time. For the complete removal of NaCl, the sample was washed several times with milli-Q water. SEM micrograph shows disappearance of octahedral crystals & only few spherical particles were visible. Washing resulted in the flakes of crystals plates in the size range between 200 - 500 nm (Figure 3.19) and also found few rhombohedral crystals in the size of 200 nm forms an aggregate (Figure 3.19). EDX analysis confirmed the absence of NaCl (Figure 3.19). XRD studies consolidated the IR data (Figure 3.20). Spectra of the washed samples showed bands corresponding to magnesian calcite and aragonite, exhibiting bands at 1826, 1456, 1070, 870, 854, and 717 cm\(^{-1}\) (Dubrawski et al. 1989, Loste et al. 2003).
Figure 3.18. SEM microphotographs of CaCO$_3$. SEM microphotographs of CaCO$_3$ obtained in the presence of poly [(ethylene-co-butylene)-b-(ethylene oxide)] in combination with magnesium on both side.

This spectrum (Figure 3.20) represents an overlap of the spectra due to aragonite phase, which is described in the literature as showing bands at 2926 / 2858 cm$^{-1}$, 2547, 1462, and 1112 cm$^{-1}$, and magnesium calcite. The slight discrepancy between values can be attribute to Mg substitution in the crystal lattice. Magnesium substitution in the calcite lattice causes increased structural disorder which results in additional weak band at 1070 cm$^{-1}$ (White et al. 1974).
Figure 3.19. SEM microphotographs of CaCO₃. After washing the sample several times in milli-Q water resulted in flakes of crystal plates and rhombohedral crystal aggregates (CaCO₃ obtained in the presence of poly [(ethylene-co-butylene)-b-(ethylene oxide)] in combination with magnesium on both side).

The intensity of these peaks increases with the quantity of Mg in the calcite structure (Loste et al. 2003). The 1070 cm⁻¹ band additionally shifts to higher frequencies with increasing Mg substitution. The 717 cm⁻¹ band (Figure 3.20) uniquely indicates calcite (the shift to longer wavenumber being due to Mg substitution).

In solutions with higher Mg to Ca ratio, the precipitation of ACC occurs (Loste et al. 2003), which subsequently transformed into crystalline CaCO₃ on ageing in solution. As mentioned before in the section 3.3.2.1, the experimental result also shows here that an incorporation of small amount of poly [(ethylene-co-butylene)-b-(ethylene oxide)] into a crystallite may create internal strain, thus
increasing solubility and the critical size required for a stable precipitate. This could be one of the reasons for the transformation of ACC to magnesian calcite and aragonite phases. The resulted magnesian calcite and aragonite phases remained stable in solution, due to the incorporation of Mg into the crystal lattice. The IR spectra were also indicative of inclusion of Mg in the calcite lattice, showing a shift in the band (Figure 3.20) at 712 (characteristic peak for calcite) from 717 cm⁻¹ (Loste et al. 2003).

![FTIR analysis](image)

**Figure 3.20. FTIR analysis.** FTIR analysis shows the presence of MgCO₃ and aragonite modifications (CaCO₃ obtained in the presence of poly [(ethylene-co-butylene)-b-(ethylene oxide)] in combination with magnesium on both side).
3.3.2.3 CaCO₃ precipitation using [(ethylene-co-butylene)-b-(ethylene oxide)] in combination with N-acetyl-L-glutamic acid

Experiments, which were carried out using surfactant poly [(ethylene-co-butylene)-b-(ethylene oxide)] in combination with N-acetyl-L-glutamic acid (by adding 20 mg of N-acetyl-L-glutamic acid to the polymer surfactant solution mix in both miniemulsions) resulted in the formation of small bundles of needle-like aggregates that were variable in length (typically < 100 nm) but highly uniform in width (25 nm) (Figure 3.21).

Figure 3.21. SEM microphotographs of CaCO₃. SEM microphotographs of CaCO₃ obtained in the presence of poly [(ethylene-co-butylene)-b-(ethylene oxide)] in combination with N-acetyl-L-glutamic acid.

EDX analysis on these bundles showed peaks for calcium, carbon and oxygen. XRD analysis of the washed samples showed sharp calcite peaks (Figure 3.22 upper), whereas FTIR data (Figure 3.22 lower) indicated that the presence of both calcite and aragonite modification due to the observation band at 2930, 2516, & 1431 cm⁻¹ for calcite and 1788, 1477, 1112, 869, 706 and 536 cm⁻¹ for aragonite. These values compare with published IR data, produced by FTIR of KBr.
pellets of 2931, 2515, & 1429 cm\(^{-1}\) for calcite and 1789, 1477, 1119, 858, and 700 cm\(^{-1}\) for aragonite (White et al. 1974).

**Figure 3.22.** XRD patterns of CaCO\(_3\). Upper) XRD patterns of CaCO\(_3\) particles obtained in the presence of poly [ethylene-co-butylene]-b-(ethylene oxide)] in combination with N-acetyl-L-glutamic acid and lower) IR spectra for the corresponding sample.
The discrepancy between XDR and IR spectroscopy methods might due to the fact that the XRD will not detect the polymorph if the sample contains less than ca.10 mass% of the according modification. FTIR was more sensitive for the identification of mineral phases.

TGA profiles of washed samples indicated that ~ 10 % of surfactant and ~ 4 % of additives were associated with the small bundles of needle-like aggregates. This was consistent with data obtained from FTIR analysis of washed and dried samples. The small bundles of needle-like aggregates were significantly more rigid and homogeneous in structure. The needle-like aggregates tended to form small bundles, presumably due to the presence of surrounding surfactant molecules. My assumption that with time, the length of the needle continued to increase with no apparent change in the width, and small bundles were observed. The needle-like calcite aggregates were stable for a long period up to several months in air.

A much wider range of morphologies is produced in the control experiment containing poly [(ethylene-co-butylene)-b-(ethylene oxide)] alone (Figure 3.14). For the additive studied here (N-acetyl-L-glutamic acid), in combination with poly [(ethylene-co-butylene)-b-(ethylene oxide)] had morphology of needle-like aggregates. Addition of N-acetyl-L-glutamic acid to the crystallising solution resulted in two effects: a transition form wider range of morphologies to a selective morphology of needle-like aggregates and the resulted aragonite phases remained stable in solution, due to the incorporation of carboxylate group into the crystal lattice and inhibited further transformation into calcite. The existing calcite phase could be probably occurred by the continuous dissolution/re-precipitation
mechanism, which occurs during washing the sample (Sawada, K. 1997).

3.4 Conclusion

The experiments demonstrate that the polybutene-succinimide pentamine, incorporation within amorphous calcium carbonate (ACC) significantly retards transformation into more stable calcite phase. Polybutene-succinimide pentamine surfactant, the poly (butene) block was expected to interact with the particle surface with the succinimide pentamine block extending into the medium. This succinimide pentamine block would be compatible with the inorganic particles as the dispersion medium, thus providing steric stabilization (Erdem et al. 2000). Amorphous calcium carbonate (ACC) to vaterite polymorphs will be changed into calcite on extended aging time through a solvent mediated process (Spanos et al. 1998) in solution. However, even though our amorphous calcium carbonate samples were aged in solution and also in dried state (after evaporation of cyclohexane) for long times (e.g. 14 days); the XRD, FT/IR, Raman and SEM analyses exhibited the same ACC phase structure and morphology. Transformation of the amorphous phase to crystalline phase was kinetically stabilized over several days, presumably due to exclusion of water molecules by the surfactant overlayer. This indicates that the ACC polymorphs can be stabilized under this experimental condition.

In contrast, analogous studies on washed ACC samples showed the presence of vaterite as well as small amount of calcite modifications. TGA profiles of the washed calcium carbonate particles indicated that surfactant molecules were intimately associated with the spherical particles. This was consistent with FTIR data of washed and dried
samples. This clearly shows that surfactant chemically adsorbs on the CaCO₃ surface, which interact with the amine end group of polybutene-succinimide pentamine. The surfactant (H-N) adsorption is explained by the change of solubility of CaCO₃ and surfactant in aqueous solution. When the pH of the solution increases the solubility of calcium carbonate in solution decreases and the solubility of surfactant increases. In pH range 9 to 11 surfactant adsorption can be expected on the surface of the calcium carbonate and at this stage vaterite polymorph is stabilized.

FT/IR and Raman spectroscopy was used to investigate the phase transformation. The result shows that the transformation from CaCO₃:H₂O to CaCO₃:6H₂O occurred after the removal of cyclohexane from the sample. This indicates that the calcium carbonate monohydrate in the miniemulsion, the approach of water molecules due to humid air is inhibited by the oil phase. It is well known that less stable form transforms readily and irreversibly into metastable vaterite via to the most stable calcite form through a solvent-mediated process (Spanos et al. 1998). A similar mechanism could happen while washing and drying the samples. We can conclude that the resulted unstable vaterite phase during washing could be transiently stabilized by the adsorbed polymer surfactant and the some of the vaterite phases which are not occluded by the polymer surfactant, could be precipitate and subsequently transforms into the most stable calcite phase.

Ostwald-ripening and solvent-mediated recrystallization process depends on the presence of a favorable difference in interface energy as a driving force (Cölfen et al. 2001). It must be underlined that calcium carbonate monohydrate (CaCO₃:H₂O) and calcium carbonate hexahydrate (CaCO₃:6H₂O) presented were stable against aging, that is,
there is no positive interface energy difference which drives the process. This goes well with the observation that stable vaterite particles can be produced in the presence of organic additives that can stabilize the vaterite surface, such as polybutene-succinimide pentamine. It is assumed that in this case the preferential adsorption of the polymer surfactant on vaterite relative to calcite can at least balance the relative loss in the crystallization energy.

Amorphous calcium carbonate (ACC) was the first phase precipitated in the control experiment containing poly [(ethylene-co-butylene)-b-(ethylene oxide)] alone. Poly [(ethylene-co-butylene)-b-(ethylene oxide)] can considerably affect crystal nucleation, often stimulating aggregation. Small concentrations of organic additives typically affect crystallization via adsorption of the active molecules at a number of growth sites on the surface of pre-critical nuclei, preventing growth beyond the pre-critical size (Meldrum et al. 2001). Incorporation of small amount of poly [(ethylene-co-butylene)-b-(ethylene oxide)] into a crystallite may create internal strain, thus increasing solubility and the critical size required for a stable precipitate. This could be one of the reasons for the transformation of ACC to more stable aragonite and calcite phases.

In biological systems, both Mg and specialized organic macromolecules may be used to stabilize less stable mineral phase [e.g., ACC] (Aizenberg et al. 2002). Because of this, experiment was carried out using poly [(ethylene-co-butylene)-b-(ethylene oxide)] in combination with magnesium chloride as a second additive; it resulted in extended plate-like crystals consisting of stacked spherical aggregates upon these crystal plates. The formation of these crystal plates can be attributed to the initial production of ACC particles
which aggregates to form an extended plate-like crystal. Transformation into vaterite and aragonite occurs by a continuous dissolution / re-precipitation mechanism (Sawada, K. 1997).

When magnesium chloride is used in both miniemulsions, it resulted in the nucleation of spherical crystals and also found several octahedral single NaCl crystals. The washed sample resulted in the disappearance of octahedral crystals and appeared in the flakes of crystals plates and also found few rhombohedral crystals containing both the MgCO$_3$ and aragonite phases. Magnesium substitution in the calcite lattice causes increased structural disorder which results in additional IR weak band at 1070 cm$^{-1}$ (White et al. 1974). The IR band at 717 cm$^{-1}$, uniquely indicates calcite (the shift to longer wavenumber being due to Mg substitution). The resulted MgCO$_3$ and aragonite phases remained stable in solution, due to the incorporation of Mg into the crystal lattice.

A much wider range of morphologies is produced in the control experiment containing poly [(ethylene-co-butylene)-b-(ethylene oxide)] alone. For the additive studied here (N-acetyl-L-glutamic acid), in combination with poly [(ethylene-co-butylene)-b-(ethylene oxide)] had morphology of needle-like aggregates. Addition of N-acetyl-L-glutamic acid to the crystallising solution resulted in two effects: a transition form wider range of morphologies to a selective morphology of needle-like aggregates and the resulted aragonite phases remained stable in solution, due to the incorporation of carboxylate group into the crystal lattice and inhibited further transformation into calcite. The existing calcite phase could be probably occurred by the continuous dissolution/re-precipitation mechanism, which occurs during washing the sample (Sawada, K. 1997).
It is difficult to fully understand the mechanism implication of the effect of the polymers surfactant over the crystallization of CaCO₃ and this will be the subject of future work. The results indicate that confinement of calcium carbonate deposition within the miniemulsion has marked influence on structure, morphology, and higher-order assembly, suggesting that further exploration of these systems should lead to new types of organized nanomaterials with complex form and architecture for potential application in diverse fields.
Chapter 4

Atomic Force Microscopy

Investigation on CaCO₃ deposits of Porcellio scaber

In this study, Atomic force microscopy (AFM) has been used to image the microstructure of the ACC (amorphous calcium carbonate) deposits of land-based woodlice (Porcellio scaber) with the dimension of 1-2 µm. We have seen that, homogenous layer (hl) consists of numerous spheres with diameters between 600 and 700 nm which reveals concentric growth pattern. The microstructure of the spheres in this region appears rougher than the surrounding material. These are mainly mineralized spheres appears to be more or less homogenous. Two different regions can be clearly differentiated by the appearance of spheres with two phase structure. The average height and RMS roughness increase with decreasing pH in a consecutive manner. The amount of decalcified minerals can be estimated in a quantitative way by the section analysis.

4.1 Introduction

4.1.1 Direction of research

Amorphous calcium carbonate (ACC) is one of the six known forms of calcium carbonate, excluding high-temperature and pressure forms. The other five crystalline, and some contain water molecules as part of their lattice structure (Lippmann et al. 1973). Calcite and aragonite are by far the most widely produced forms of calcium carbonate in biology.
(Lowenstam et al. 1989). Vaterite and amorphous calcium carbonate, although less stable, are formed by a limited number of organisms. A land-based woodlouse (*Porcellio scaber*) is the best example for the appearance of ACC in the sternal CaCO₃ deposits (Figure 4.1).

**Figure 4.1. CaCO₃ deposits of Porcellio scaber.** The sternal CaCO₃ deposits of *Porcellio scaber* in the late premolt stage. Light micrographs of the deposits outlining deposits of the sternites 2-4. The smaller first sternite is hidden by the head. Source: Helge Fabritius and A. Ziegler J. Struct. Biol. 142 (2003) 281-291

These isopods use CaCO₃ as a mineral for hardening their cuticle so as to provide stability for their exoskeletons (Passano et al. 1960). This CaCO₃ deposits which contain ACC surrounded by organic envelope. The cuticle has to be regularly replaced by a larger one because it cannot expand during growth of the animal. During this development and associated growth force (molting process) calcium which is left within the old cuticle is lost and must be replaced to mineralize the new cuticle. In marine crustaceans lost calcium can be
replaced from seawater in which calcium ions are readily available. The terrestrial isopods do not have this possibility to replace lost calcium. Therefore they have to store and recycle calcium by taking up Ca\(^{2+}\) from the old cuticle. For this cycle these animals store CaCO\(_3\) as an amorphous compound between the cuticle and epithelium of the first four anterior sternites (Ziegler et al. 1994) (Figure 4.2).

**Figure 4.2.** Molting process. Formation and reabsorption of the sternal CaCO\(_3\) deposits. Source: A. Ziegler et al. Cell Calcium, 2002, 32(3), 131-141

Previous studies (Fabritius et al. 2003) revealed that the deposits consist of two distinct regions, a distal opaque region consisting of spherules and a proximal glassy layer of homogeneous nature. The spherular region is further divided into a distal spherular layer (dsl) with small, partially fused spherules and a proximal spherular layer (psl) consisting of free spherules (Figure 4.3), the diameters of which increase from distal to proximal. Investigations (Ziegler, A. 1994, 1997,
2002 and Becker et al. 2003) by scanning electron microscopy (SEM), light microscopy and X-ray microprobe analysis have shown that in all three layers CaCO₃ deposits of Porcellio scaber are built by numerous spherules and associated with an organic matrix consisting of radial and concentric elements, suggesting that the organic components functions as a template for spherule formation.

Figure 4.3. Structure of the sternal CaCO₃ deposits. Cleaved surface of untreated deposits showing that the material within hl and the spherules within psl and dsl appear solid. Source: H. Fabritius and A. Ziegler J. Struct. Biol. 142 (2003) 281.

ACC is the only form of calcium carbonate that is isotropic in polarized light and does not diffract X-rays; hence it is described as being amorphous. It is well known that amorphous (Brečević et al. 1989) CaCO₃ is more soluble compared with crystalline phases due to its large surface area. This may be an advantage when frequent replacement of the mineral is required. Most of the crustaceans have
mineralized exoskeletons (or cuticles) that are cyclically renewed with endogenous calcium carbonate.

Recently (Fabritius et al. 2003) it is reported that the formation of the spherules takes place in a specialized aggregation zone, in which 10 to 30 nm thick granules form agglomerations that then increase in size to form spherules that reveals a concentric growth pattern. During degradation of spheres, a maximum of surface area is exposed by differential stabilization of amorphous CaCO$_3$ by components of the organic matrix. The large surface increases the speed of Ca$^{2+}$ and HCO$_3^-$ mobilization during the quick degradation of the deposits. Detailed information about the formation, composition, structure and degradation of the sternal calcium carbonate deposits were based on scanning electron microscopy (SEM), light microscopy and X-ray microprobe analysis. Therefore in this study, the microstructure of the sternal calcium carbonate deposits is investigated using the Atomic Force Microscopy (AFM).

Scanning Force Microscopy (SFM), such as the Atomic Force Microscopy (AFM) in contact mode (Binnig et al. 1986) or tapping mode (Hansma et al. 1993) was used successfully to analyze inorganic (Guntherold et al. 1995), organic and biological surfaces (Marti et al. 1993, Radmacher et al. 1992, Grimelec et al. 1995) from the micrometric level to the atomic resolution (Ohnesorge et al. 1993). Many authors (Shindo et al. 1992, 1993, Bosbach et al. 1994, 1994a, Hall et al. 1996, Shindo et al. 1996) have explored the ability of the Atomic Force Microscopy to study the process of growth and dissolution of the inorganic crystals. So far dissolution behavior of biogenic ACC has not been investigated using Atomic Force Microscopy (AFM). AFM, which reveals the local structure of the surface, would be an appropriate
method to study the dissolution mechanism of amorphous calcium carbonate at a microscopic level. In the present study the terrestrial isopod *Porcellio scaber* is used as a model and it is an interesting example to investigate the dissolution behaviors of ACC by AFM.

4.1.2 Material and Methods

4.1.2a Animals

*Porcellio scaber* were collected from local populations and reared in plastic containers filled with moist soil and bark. Potatoes, carrots and dry oak leaves were offered for food. Animals in the late premolt stage were determined by the optical appearance of the sternal CaCO$_3$ deposits according to Messner (1965). Only specimens with fully developed deposits were used for the experiments (Courtesy: PD Dr. Ziegler for providing animals).

4.1.2b Sample preparation for dissolution studies

For the sequential dissolution and subsequent SFM imaging the calcium carbonate deposits of *P. scaber* were glued on special PMMA sample holder (Figure 4.4). After this, the samples were mounted on an ultra microtome (Leica Ultracut UCT) and a block face was trimmed by taking off 0.5 µm thick sagittal sections using dry glass knifes. Diamond knifes were used to polish the surface of the block face by advancing the knife successively 15 times each by 70 nm, 30, 20, 10 and 5 nm. That way we obtained flat and smooth block faces with a minimum of knife marks (Courtesy: PD Dr. Ziegler for sample preparation). Thereafter the specimens were decalcified in different pH (8, 9, & 10) buffer solutions with different time scale and were dehydrated in 100% methanol.
Atomic Force Microscopic Investigation on CaCO$_3$ deposits of *Porcellio scaber*

4.1.3 The dynamic SFM mode

In order to avoid the large frictional forces present in contact mode, we prefer to use the dynamic mode like tapping mode™ in air. The cantilever oscillates at an adjustable amplitude and resonance frequency which allows for the small silicon tip to be out of contact with the surface during most of the cycle (den Boef *et al.* 1991, Annis *et al.* 1993, and Warmack *et al.* 1994). To minimize the adhesion of the tip with the sample, the driving frequency was set just above the resonance (Anczykowski *et al.* 1996). To investigate the microstructure of the ACC deposits of *Porcellio scaber*, we used smooth surface of the calcium carbonate deposits prepared by cryo microtome. Phase

---

**Figure 4.4. Set-up for SFM experiments.** For the sequential dissolution and subsequent SFM imaging the calcium carbonate deposits of *P.scaber* are glued on special PMMA sample holder (Courtesy: Dr. S. Hild).
imaging in tapping mode\textsuperscript{TM} under ambient conditions was chosen to investigate the microstructure of the ACC deposits. In the SFM specific interactions between a small silicon tip and the surface are measured with this technique, enables to image simultaneously the surface topography of non-conducting materials with high resolution in the Z-range and local material properties like the local compliance. Dynamically techniques such as the tapping mode\textsuperscript{TM} provides the possibility to image sensitive, soft surfaces like biological samples without destructive shear forces. In tapping mode\textsuperscript{TM}, phase shift as a function of the free amplitude and the set point tapping amplitude can be used to investigate (Magonov et al. 1997, Sarid et al. 1991) the local mechanical properties of the sample. This enables to differentiate the soft organic regions from hard inorganic ones. Variations in surface roughness have been used to study the pH-dependence of the dissolution of ACC. Cryo microtome cuts of homogenous layer (hl) are extremely flat and show some circular features like intersected spheres with mean surface roughness in the range 2 to 3 nm were used to investigate pH dependence of the dissolution of ACC.

4.1.4 Roughness Analysis

For SFM imaging no special sample treatment like evaporating metals to the surface is necessary. For this purpose sequential measurement at the same surface becomes possible. For instance, by repeated immersion of the samples in a buffer solution of different pH-values the influence of different pH-values in the dissolution of ACC dependent on the immersion time can be estimated. Variations in surface roughness have been used to study the pH-dependence of the dissolution of ACC.
Figure 4.5. **Effect of Methanol.** AFM image of the surface of hl-layer, recorded before and after treatment with methanol.

Microtome cuts of the glassy region are extremely flat and show some circular features likely intersected spheres. The mean surface roughness is in the range of about 2 to 3 nm. Immersing the sample with buffer solution with different pH-values from 8 - 10 with different time scales and taken SFM topography images after this procedure.
reveals single spheres with a diameter of about 1 \( \mu \text{m} \). These spheres show a core-shell structure. The amount of etched material can be characterized by the increasing mean roughness. Whereas for samples immersed in buffer solution for 20 second with pH 8 a mean roughness of about 38 nm can be found whereas the samples immersed in buffer solution for 20 second with pH 10 exhibit a mean roughness of only 11 nm. This reflects a pH-dependence of the dissolution of ACC which is similar to the behavior of crystalline CaCO\(_3\). The repeated immersion of the cryo cut sample with a buffer solution of pH 10 using immersion times of about 2 minutes for each step enables to follow the development of the spherical structures.

4.2 Results

4.2.1 Effect of Methanol

In order to clean the sternal plate and for terminating dissolution process, we used 100\% methanol. To test the effect of methanol on the phase transformation of CaCO\(_3\) deposits, AFM is used to analysis the change in average height of the \( hl \)-layer before and after treatment with methanol. Figure 4.5 shows a typical height AFM image of the surface of \( hl \)-layer, recorded in air with scan size of 10 \( \mu \text{m} \) x 10 \( \mu \text{m} \) before and after treatment with methanol. It can be seen that the exposure of knife polished \( hl \)-layer of the deposits to 100\% methanol for five minutes leads to the removal of smear, revealing that there is no significant differences in RMS roughness (Figure 4.5). This was confirmed by taking section analysis across the concentric layers (Figure 4.6). The results indicate the surface appeared to be static: the surface showed the similar height and surface roughness before and after treatment with methanol.
4.2.2 Concentric Growth Mechanism

Atomic force micrographs of homogenous layer (hl) as well as in the adjacent psl-layer illustrate the various features of the growth and arrangements of the minerals (Figure 4.7). The growth patterns of the mineral depend on the surrounded organic matrix. With the sequential decalcification using various pH buffer solutions at different time scale, the overall organization of the matrix gradually changes its appearance. This is demonstrated for hl-layer as well as in the adjacent psl-layer (Figure 4.7-4.10).

Figure 4.6. Section analysis. Change in step height determined for hl-layer before and after treatment with methanol.
Figure 4.7. The growth patterns of the mineral. SFM image of the topography of knife-polished calcium carbonate deposits show inhomogeneous structures in the hl-layer as well as in the adjacent psl-layer.

4.2.2a Sequential decalcification at pH 8, 9 and 10 with different time scales.

Dissolution studies were undertaken to investigate the influence of pH 8, 9, and 10 on the solubility of ACC with different time scales (Figure 4.8). It shows partly decalcified hl-layer and the adjacent psl-layer with an increasing surface roughness ~ 300, 400 and 500 nm for the time scale 40, 60 and 100 seconds respectively.
Sequential decalcification at pH = 10 for different time scales

**Figure 4.8. Influence of pH 10.** SFM image of the topography of CaCO$_3$ deposits show inhomogeneous structures in the hl-layer as well as in the adjacent psl-layer after decalcification at pH 10 with different time scale.

**Figure 4.9. Line section analysis.** Section analysis shows inhomogeneous dissolution of the deposits. Higher areas indicate reduced dissolution. Differences in the mean sample height were used to quantify the dissolution process.
Line section analysis shows inhomogeneous dissolution of the deposits (Figure 4.9). Higher area indicates reduced dissolution. Differences in the mean sample height were used to quantify the dissolution process. After decalcification of psl-layer at pH 10 for different time scales (Figure 4.10) the overall organization of the deposits changes its appearances. The appearance, however, is as follows: the partially decalcified psl-layer contains numerous onion-like spheres with diameters between 600 and 700 nm which reveals a two phase structure with increase in surface roughness with decrease in pH (Figure 4.10).

![Figure 4.10. Partially decalcified psl-layer. SFM topography image of the CaCO$_3$ deposits of P.scaber after sequential decalcification at pH 10 with different time scales.](image)

Two different regions can be clearly differentiated by the appearance of spheres with two phase structure (Figure 4.8 - 4.10). Knife polished sagittal faces of the hl-layer treated with buffer (pH 9) solution for different time scale reveals that it is built of several concentric layers that originate from spherules in the transition zone between psl and hl (Figure 4.11).
Figure 4.11. Partially decalcified hl-layer. SFM topography image of the CaCO$_3$ deposits of P.scaber after sequential decalcification at pH 9 for different time scales.

Sequential decalcification at pH = 9 for different time scales

Figure 4.11 shows that by increasing dissolution time, the concentric growth development become more apparent with an increased surface roughness. The interesting result of the present study is that despite their structural differences the psl & hl-layers of the sternal deposits contain virtually similar matrix components and this in good agreement with the observation by Fabritius et al (Fabritius et al. 2005).

In the transition zone between psl and hl, the space between the most proximal spherules are filled by concentric and radial matrix elements similar to those in more central regions of the spherules. This results in polyhedral structures similar to those found in fused dsl spherules (Fabritius et al. 2005). It is demonstrated (Fabritius et al. 2005) that the thickness and structure of the concentric layers equal those of the spherules in dsl and hl. With increasing distance from psl some of the stacks of matrix layer become broader from distal to proximal, while others are narrowing constantly until they discontinue (Figure 4.12).
Atomic Force Microscopic Investigation on CaCO$_3$ deposits of *Porcellio scaber*

Figure 4.12. Ultrastructure of homogeneous layer. Ultrastructure of the organic matrix within the homogeneous layer (hl). Competitive growth pattern of hl segments originating from psl spherules. The concentric lamellae are connected by radial strands extending from spherules in the transition between hl and the proximal spherular layer. Source: H. Fabritius and A. Ziegler J. Struct. Biol. 142 (2003)

The study on knife polished faces of hl treated with buffered (pH 8) aqueous solutions after sequential decalcification for different time scales indicates that the surface roughness increases with increasing dissolution time (Figure 4.13).

With longer exposure times (2 min 40sec) the development of concentric rings become more and more pronounced (Figure 4.13) until several; bright spots appear with diameter between 10 and 40 nm (Figure 4.14). The concentric rings are between 20 and 50 nm thick. Fabritius and coworkers (Fabritius *et al.* 2005) had shown that distance between concentric rings is about 100 nm with the distance between

- Dissolution in 0.1 M EDTA for 10 min.
- 2.5% Glutaraldehyde is used as protein fixation.
the innermost ring structures to the first concentric ring being somewhat smaller.

**Sequential decalcification at pH = 8 for different time scales**

![Sequential decalcification at pH = 8 for different time scales](image)

**Figure 4.13. Sequential decalcification at pH 8.** SFM topography image of the CaCO₃ deposits after sequential decalcification at pH 8 with different time scales.

The repeated immersion of the sample with a buffer solution of pH 8 using immersion times of about one hour enables to see some development of crystal-like appearance with RMS roughness of about 177.5 nm (Figure 4.14) and I assume that the amorphous modification transforms to crystalline modification or it could be recrystallized (Ostwald ripening). Why this crystal-like appearance occurs is not yet
clear at the present time. Sequential decalcification of hl layer at pH 8 reveals several concentric rings and it could be able to count up to seven concentric rings (Figure 4.15) from central sphere to till more pronounced rings, which is in good agreement with Fabritius et al (Fabritius et al. 2005) findings.

Sequential decalcification at pH = 8 for 2 min 40 sec

![Sequential decalcification at pH = 8 for 2 min 40 sec](image)

Decalcification at pH = 8 after 1 hour

![Decalcification at pH = 8 after 1 hour](image)

**Figure 4.14. Development of bright spots.** SFM topography image of the CaCO₃ deposits after sequential decalcification at pH 8 reveals the development of bright spots and face difficult to image the sample further decalcification for 1 hour.

The average height and RMS roughness increases with decreasing pH in a consecutive manner (Figure 4.16). Figure 4.16 shows that by decreasing pH, the amount of dissolved materials increases.
Figure 4.15. **Seven concentric rings.** SFM topography image of the CaCO₃ deposits after sequential decalcification at pH 8 reveals several concentric rings and it could be able to count up to seven concentric rings from central sphere to till more pronounced rings.

Figure 4.16. **Amount of dissolved materials.** Change in mean sample height determined for different pH values for the time scale 40 seconds. It reveals that by decreasing pH the amount dissolved materials increases. (Courtesy: Dr. Hild for preparing graph from AFM images which I performed)
Figure 4.17 shows the change in mean height determined for different pH 8, 9, 10, & 12 values and decalcification follows an exponential behavior. This suggests that the ACC stabilizing properties of the matrix are by themselves pH dependent. Together with the findings of Fabritius and coworkers (Fabritius et al. 2005), the results leads to a better understanding that the differences in the solubility of amorphous calcium carbonate within individual spherules are caused by variations in the stabilizing properties of the organic matrix.

\[ f(t) = A(1 - \exp(-B \cdot t)) \]

<table>
<thead>
<tr>
<th>pH</th>
<th>A / nm</th>
<th>B / min(^{-1})</th>
</tr>
</thead>
<tbody>
<tr>
<td>8</td>
<td>284</td>
<td>0.017</td>
</tr>
<tr>
<td>9</td>
<td>266</td>
<td>0.012</td>
</tr>
<tr>
<td>10</td>
<td>251</td>
<td>0.011</td>
</tr>
</tbody>
</table>

**Figure 4.17. Differences in the solubility.** The dissolution behavior follows an exponential function in which the maximum mean sample height varies with pH. This suggests that the ACC stabilizing properties of the matrix are by themselves pH dependent. (Courtesy: Dr. Hild for preparing graph from AFM images which I performed)

4.3 Discussion

Exposure of knife polished sagittal faces of the CaCO\(_3\) deposits to aqueous decalcification solutions such as 10 mM buffer (pH 10) for 20 second leads to dissolution of rather unstable material within the deposits, revealing new aspects of CaCO\(_3\) deposit organization. In, psl, this exposes a polyhedral structure of fused spherules (Figure 4.8 -
4.10). Part of the spherules within psl dissolve first with a region about 200 nm around their centre leaving a core structure (dark region) Figure 4.10. This is in good agreement with the observation by Fabritius and coworkers (Fabritius et al. 2005).

![Image of Topography and Phase](image)

**Figure 4.18. Dark and bright concentric layers.** Change in mean sample height determined for different pH values and decalcification times follow an exponential behavior. Here the lower phase shift indicates soft regions and larger phase shift indicates harder materials. (Courtesy: Dr. Hild for section analysis from AFM images which I performed)

On sequential decalcification for different times scale reveals the substructure of the organic matrix, which is composed of several concentric layers. The concentric layers are between 20 and 50 nm thick
and contain particles with diameters between 10 and 40 nm. Similar results were observed by Fabritius and coworkers (Fabritius et al. 2005) using electron microscopical examination. Figure 4.18 shows some dark and bright concentric layers which indicates us some hint that the matrix is composed of several materials which are having differences in their solubility. The change in mean height for different pH and decalcification times follow an exponential behavior as shown in Figure 4.18. Here the lower phase shift indicates some soft regions and larger phase shift show harder materials. The concentric growth layers in $hl$, which arise from spherical structures, have distances between one another similar to the distances between concentric layers in spherules. Together with previous investigation (Fabritius et al. 2005), these results make it evident that all three layers of the sternal ACC-deposits arise from the concentric growth of ACC-spherules with striking similarities in the architecture of their organic matrix.

4.4 Conclusion

This chapter presents a preliminary result of ultrastructural investigation of the organic matrix of the sternal CaCO$_3$ deposits of Porcellio scaber by using Atomic force microscopy (AFM). It reveals that the ultrastructure of the organic matrix is similar within each of the three layers of the CaCO$_3$ deposits. Together with previous investigations on deposit composition (Ziegler 1994, Becker et al. 2003), and also the investigation on architecture of the organic matrix in the sternal CaCO$_3$ deposits of Porcellio scaber (Fabritius et al. 2005), the results lead to a better understanding of matrix function. These studies suggest that differences in the solubility of amorphous calcium carbonate within individual spherules are caused by variations in the stabilizing properties of the organic matrix.
Chapter 5

Summary and Outlook

The process of Biomineralization and Biomimicry is well documented and studies on Calcium Carbonate precipitation from aqueous solutions have been performed by a large number of authors. Literature relevant to this work has been gathered and summarized in chapter 1.

In chapter 2, I demonstrate the effect of water-soluble polymers on the crystallization of calcium carbonate is reported. The polymers used were acrylamide based on acrylamido methyl propane sulphonic acid with varying monomer compositions and monomeric units. For the desired purpose of ion binding, several low molecular weight additives such as simple amino acids, peptides, proteins, inorganic salts were also examined in combination with polymers. Crystallization began by mixing together, with or without a polymer or other additive, calcium chloride and sodium/potassium carbonate. The first crystals to form in most cases were vaterite, possibly with a very small amount of calcite. Subsequent behavior depended on the polymer or other additive present. With most additives, the vaterite transformed within ca. 1 h to calcite, probably by dissolution and recrystallization.

The observed aggregate shape and the selectivity for the crystal modification can be attributed to the co-operative effect of pre-adsorbed additives and the functionalities (amide, carboxylic, sulphonic acid) of the terpolymer. The interaction between various
functional groups and ionic species present in CaCO₃ and stereochemical matching between the oriented functional groups and the crystal plane are fundamentally responsible for the stabilization of specific aggregates. Nucleation of the calcite \{110\} faces is favored by carboxylate anions because the bidentate motif mimics the carbonate stereochemistry exposed on this crystal surface. On the other hand, the nucleation of the \{001\} face is induced by sulfate head groups because the tridentate arrangement simulates the oxygen positions of carbonate anions lying parallel to this crystal surface (Heywood et al. 1994). Thus it appears most likely that the initiation and nucleation of various forms of crystal modifications on the terpolymer employed occurs through binding of calcium ions at the ionizable carboxylic and sulphonic groups.

Summary of FTIR analysis shows that in all samples methylene symmetric and antisymmetric vibrations have reduced marginally in intensity following Ca²⁺ incorporation. This indicates considerable reorganization in the polymer, possibly due to randomization of hydrocarbon chain orientation around the CaCO₃ crystals. This is supported by AFM results. In some cases we could able to see from IR spectra almost complete disappearance of the methylene symmetric and antisymmetric vibration bands after CaCO₃ nucleation indicates considerable reorganization in the polymer chemical structure, possibly due to the randomization of several low molecular weight additives or macromolecular chain orientation around the CaCO₃ crystals. After entrapment of additive, the FTIR absorption band for the sulphate group from polymer at 1046 cm⁻¹ has got shifted clearly indicating that the additives complexes the sulfonate group of the terpolymer.
The stability of the polyelectrolyte solution depends on the counterion valency and the characteristics of the polymer: nature of backbone (hydrophobic, hydrophilic, rigid, and flexible), distance between charged groups, and the nature of charged groups (carboxylate, sulfonate, carbonyl, and amide). AFM imaging on terpolymer surface, in presence of divalent cations and several low molecular weight additive containing charged groups, were established. In presence of different salt solutions, we could able to see that, the variation of the terpolymer conformation. Entrapment of L-glutamine, N-acetyl-L-glutamic acid and glutathione in the terpolymer, has enable us to visualize in situ the interaction of low molecular weight additives on terpolymer, resulting in compaction of macromolecular coils caused by deterioration of thermodynamic quality of a solvent. By this reason at very high salt concentration polymers can loose their solubility and precipitate from solution. These images make possible direct, visual observation of micro-environmental changes brought about by the adsorption of additives (acidic peptides / simple amino acids / magnesium ions) play a significant role in inducing the deposition of different CaCO₃ crystal modifications.

In chapter 3, I will describe a versatile method for the nucleation of calcium carbonate aggregates with different morphologies and textures fabricated by the miniemulsion technique. Inverse miniemulsions can be prepared by emulsifying an aqueous inorganic salt solution in a continuous oil phase. The solution is dispersed in the continuous phase by means of an adequate amount of surfactant by fast stirring and the efficient use of ultrasonication. Here small droplets in the size range of 50-500 nm with sufficient stability are created. For
the preparation one inverse miniemulsion consisting of Ca\(^{2+}\) ions containing droplets and another miniemulsion consisting of CO\(_{3}^{2-}\) ions containing droplets are prepared. Adding these two miniemulsions while applying high shear lead to the CaCO\(_{3}\) nanoparticles.

The miniemulsion experiments demonstrate that the polybutene-succinimide pentamine, incorporation within amorphous calcium carbonate (ACC) significantly retards transformation into more stable calcite phase. Polybutene-succinimide pentamine surfactant, the poly (butene) block was expected to interact with the particle surface with the succinimide pentamine block extending into the medium. This succinimide pentamine block would be compatible with the inorganic particles as the dispersion medium, thus providing steric stabilization (Erdem et al. 2000). Amorphous calcium carbonate (ACC) to vaterite polymorphs will be changed into calcite on extended aging time through a solvent mediated process (Spanos et al. 1998) in solution. However, even though our amorphous calcium carbonate samples were aged in solution and also in dried state (after evaporation of cyclohexane) for long times (e.g. 14 days); the XRD, FT/IR, Raman and SEM analyses exhibited the same ACC phase structure and morphology. Transformation of the amorphous phase to crystalline phase was kinetically stabilized over several days, presumably due to exclusion of water molecules by the surfactant overlayer. This indicates that the ACC polymorphs can be stabilized under this experimental condition.

In contrast, analogous studies on washed ACC samples showed the presence of vaterite as well as small amount of calcite modifications. TGA profiles of the washed calcium carbonate particles indicated that surfactant molecules were intimately associated with the spherical
particles. This was consistent with FTIR data of washed and dried samples. This clearly shows that surfactant chemically adsorbs on the $\text{CaCO}_3$ surface, which interact with the amine end group of polybutene-succinimide pentamine. The surfactant (H-N) adsorption is explained by the change of solubility of $\text{CaCO}_3$ and surfactant in aqueous solution. When the pH of the solution increases the solubility of calcium carbonate in solution decreases and the solubility of surfactant increases. In pH range 9 to 11 surfactant adsorption can be expected on the surface of the calcium carbonate and at this stage vaterite polymorph is stabilized.

FT/IR and Raman spectroscopy was used to investigate the phase transformation. The result shows that the transformation from $\text{CaCO}_3\cdot\text{H}_2\text{O}$ to $\text{CaCO}_3\cdot6\text{H}_2\text{O}$ occurred after the removal of cyclohexane from the sample. This indicates that the calcium carbonate monohydrate in the miniemulsion, the approach of water molecules due to humid air is inhibited by the oil phase. It is well known that less stable form transforms readily and irreversibly into metastable vaterite via to the most stable calcite form through a solvent-mediated process (Spanos et al. 1998). A similar mechanism could happen while washing and drying the samples. We can conclude that the resulted unstable vaterite phase during washing could be transiently stabilized by the adsorbed polymer surfactant and the some of the vaterite phases which are not occluded by the polymer surfactant, could be precipitate and subsequently transforms into the most stable calcite phase.

Ostwald-ripening and solvent-mediated recrystallization process depends on the presence of a favorable difference in interface energy as a driving force (Cölfen et al. 2001). It must be underlined that calcium carbonate monohydrate ($\text{CaCO}_3\cdot\text{H}_2\text{O}$) and calcium carbonate
hexahydrate (CaCO$_3$·6H$_2$O) presented were stable against aging, that is, there is no positive interface energy difference which drives the process. This goes well with the observation that stable vaterite particles can be produced in the presence of organic additives that can stabilize the vaterite surface, such as polybutene-succinimide pentamine. It is assumed that in this case the preferential adsorption of the polymer surfactant on vaterite relative to calcite can at least balance the relative loss in the crystallization energy.

Amorphous calcium carbonate (ACC) was the first phase precipitated in the control experiment containing poly [(ethylene-co-butylene)-b-(ethylene oxide)] alone. Poly [(ethylene-co-butylene)-b-(ethylene oxide)] can considerably affect crystal nucleation, often stimulating aggregation. Small concentrations of organic additives typically affect crystallization via adsorption of the active molecules at a number of growth sites on the surface of pre-critical nuclei, preventing growth beyond the pre-critical size (Meldrum et al. 2001). Incorporation of small amount of poly [(ethylene-co-butylene)-b-(ethylene oxide)] into a crystallite may create internal strain, thus increasing solubility and the critical size required for a stable precipitate. This could be one of the reasons for the transformation of ACC to more stable aragonite and calcite phases.

In biological systems, both Mg and specialized organic macromolecules may be used to stabilize less stable mineral phase [e.g., ACC] (Aizenberg et al. 2002). Because of this, experiment was carried out using poly [(ethylene-co-butylene)-b-(ethylene oxide)] in combination with magnesium chloride as a second additive; it resulted in extended plate-like crystals consisting of stacked spherical aggregates upon these crystal plates. The formation of these crystal
plates can be attributed to the initial production of ACC particles which aggregates to form an extended plate-like crystal. Transformation into vaterite and aragonite occurs by a continuous dissolution / re-precipitation mechanism (Sawada, K. 1997).

When magnesium chloride is used in both miniemulsions, it resulted in the nucleation of spherical crystals and also found several octahedral single NaCl crystals. The washed sample resulted in the disappearance of octahedral crystals and appeared in the flakes of crystals plates and also found few rhombohedral crystals containing both the MgCO₃ and aragonite phases. Magnesium substitution in the calcite lattice causes increased structural disorder which results in additional IR weak band at 1070 cm⁻¹ (White et al. 1974). The IR band at 717 cm⁻¹, uniquely indicates calcite (the shift to longer wavenumber being due to Mg substitution). The resulted MgCO₃ and aragonite phases remained stable in solution, due to the incorporation of Mg into the crystal lattice.

A much wider range of morphologies is produced in the control experiment containing poly [(ethylene-co-butylene)-b-(ethylene oxide)] alone. For the additive studied here (N-acetyl-L-glutamic acid), in combination with poly [(ethylene-co-butylene)-b-(ethylene oxide)] had morphology of needle-like aggregates. Addition of N-acetyl-L-glutamic acid to the crystallising solution resulted in two effects: a transition form wider range of morphologies to a selective morphology of needle-like aggregates and the resulted aragonite phases remained stable in solution, due to the incorporation of carboxylate group into the crystal lattice and inhibited further transformation into calcite. The existing calcite phase could be probably occurred by the continuous
dissolution/re-precipitation mechanism, which occurs during washing the sample (Sawada, K. 1997).

It is difficult to fully understand the mechanism implication of the effect of the polymers surfactant over the crystallization of CaCO₃ and this will be the subject of future work. The results indicate that confinement of calcium carbonate deposition within the miniemulsion has marked influence on structure, morphology, and higher-order assembly, suggesting that further exploration of these systems should lead to new types of organized nanomaterials with complex form and architecture for potential application in diverse fields.

Chapter 4 presents a preliminary result of ultrastructural investigation of the organic matrix of the sternal CaCO₃ deposits of Porcellio scaber by using Atomic force microscopy (AFM). We have seen that, homogenous layer (hl) consists of numerous spheres with diameters between 600 and 700 nm which reveals concentric growth pattern. The microstructure of the spheres in this region appears rougher that the surrounding material. These are mainly mineralized spheres appears to be more or less homogenous. Two different regions can clearly differentiate by the appearance of spheres with two phase structure. The average height and RMS roughness increase with decreasing pH in a consecutive manner. The amount of decalcified minerals can be estimated in a quantitative way by the section analysis.

The result shows that the ultrastructure of the organic matrix is similar within each of the three layers of the CaCO₃ deposits. Together with previous investigations on deposit composition (Ziegler 1994, Becker et al. 2003), and also the investigation on architecture of the organic matrix in the sternal CaCO₃ deposits of Porcellio scaber (Fabritius et al. 2005), the results lead to a better understanding of
matrix function. These studies suggest that differences in the solubility of amorphous calcium carbonate within individual spherules are caused by variations in the stabilizing properties of the organic matrix.
# List of Figures and Tables

1.1. The sternal CaCO$_3$ deposits of *Porcellio scaber* ........................................ 3  
1.2. Ultrastructure of the organic matrix......................................................... 6  
1.3. Competitive growth pattern................................................................. 7  
1.4. Spatial charge distributions of functional groups ................................. 8  
1.5. Diagrammatic representation of the activation energies....................... 11  
1.6. Epitaxy in biomineralization................................................................. 12  
1.7. Intact coccosphere of *E. huxleyi*............................................................. 14  
1.8. Ostwald-Freundlich relation...................................................................... 25  
1.9. The different forms of Nucleation............................................................ 26  
1.10. Gibbs free energy of a nucleation............................................................ 27  
1.11. Rate of nucleation...................................................................................... 29  
1.12. Supersaturation Zone.................................................................................. 30  
1.13. Nucleation rate.......................................................................................... 32  
1.15. Ionic crystal growth-II .............................................................................. 36  
1.16. Ionic crystal growth-III.............................................................................. 36  
1.17. Ionic crystal growth-IV.............................................................................. 36  
1.18. Phase transformation.................................................................................. 38  
1.19. Relative growth rate versus dimensionless impurity.............................. 42  
2.1. Chemical structure of Polymers.................................................................. 57  
2.2. Double decomposition precipitation method.............................................. 59  
2.3. Chemical structure of Additives................................................................. 60
2.4. SEM micrographs of CaCO$_3$ crystals.................................62
2.5. SEM micrographs of CaCO$_3$ crystals.................................64
2.6. SEM micrographs of CaCO$_3$ crystals.................................65
2.7. Summary of the experimental results.................................66
2.8. SEM micrographs of CaCO$_3$ crystals.................................68
2.9. SEM micrographs of CaCO$_3$ crystals.................................69
2.10. SEM micrographs of CaCO$_3$ crystals.................................70
2.11. Summary of the experimental results.................................71
2.12. SEM micrographs of CaCO$_3$ crystals.................................72
2.13. Summary of the experimental results.................................73
2.14. SEM micrographs of CaCO$_3$ crystals.................................74
2.15. SEM micrographs of CaCO$_3$ crystals.................................75
2.16. SEM micrographs of CaCO$_3$ crystals.................................76
2.17. Summary of the experimental results.................................77
2.18. SEM micrographs of aragonite hollow crystals....................79
2.19. SEM micrographs of CaCO$_3$ crystals.................................80
2.20. AFM to examine the effects of polymer /poly-Asp.............81
2.21. AFM image of aragonite crystals.......................................82
2.22. FTIR Results.................................................................85
2.23. SEM micrographs of CaCO$_3$ crystals.................................87
2.24. SEM micrographs of CaCO$_3$ crystals.................................87
2.25. Summary of the experimental results.................................88
2.26. SEM micrographs of CaCO$_3$ crystals.................................89
2.27. Summary of the experimental results.................................90
2.28. SEM micrographs of CaCO₃ crystals........................................92
2.29. Summary of the experimental results.................................93
2.30. SEM micrographs of CaCO₃ crystals........................................94
2.31. Summary of the experimental results.................................95
2.32. SEM micrographs of CaCO₃ crystals........................................97
2.33. Summary of the experimental results.................................98
2.34. Summary of XRD analysis..................................................100
2.35. Summary of XRD analysis..................................................101
2.36. Raman analysis...............................................................101
2.37. Summary of Raman analysis...............................................102
2.38. AFM analysis.................................................................105
2.39. AFM analysis.................................................................106
2.40. AFM analysis.................................................................107
3.1. The miniemulsion principle..................................................115
3.2. Synthesis of calcium carbonate nanoparticles.......................118
3.3. Suppression of Ostwald ripening..........................................120
3.4. Chemical structure of surfactants.........................................121
3.5. Transport Mechanism........................................................123
3.6. Phase separation..............................................................127
3.7. TEM image of an ACC.......................................................127
3.8. SEM micrographs of ACC...................................................128
3.9. XRD patterns of CaCO₃.......................................................129
3.11. Summary of the experimental results...............................131
3.12. Raman analysis show the presence of ACC....................132
3.13. TGA profile and FTIR spectra recorded from CaCO3 particles..135
3.14. SEM micrographs of CaCO3..............................................143
3.15. FTIR spectra & TGA recorded from CaCO3......................144
3.16. SEM micrographs of CaCO3..............................................147
3.17. FTIR spectra recorded from CaCO3.................................148
3.18. SEM micrographs of CaCO3..............................................150
3.19. SEM micrographs of CaCO3..............................................151
3.20. FTIR analysis.................................................................152
3.21. SEM micrographs of CaCO3..............................................153
3.22. XRD patterns of CaCO3..................................................154
4.1. CaCO3 deposits of Porcellio scaber.................................162
4.2. Molting process...............................................................163
4.3. Structure of the sternal CaCO3 deposits.........................164
4.4. Set-up for SFM experiments..............................................167
4.5. Effect of Methanol............................................................169
4.6. Section analysis...............................................................171
4.7. The growth patterns of the mineral.................................172
4.8. Influence of pH 10.............................................................173
4.9. Line section analysis........................................................173
4.10. Partially decalcified psl-layer..........................................174
4.11. Partially decalcified hl-layer............................................175
4.12. Ultrastructure of homogeneous layer..............................176
List of Figures and Tables

4.13. Sequential decalcification at pH 8...................................................177
4.15. Seven concentric rings.................................................................179
4.16. Amount of dissolved materials......................................................179
4.17. Differences in the solubility..............................................................180
4.18. Dark and bright concentric layers..................................................181

List of Tables

1.1. Biological archetypes........................................................................16
2.1. FTIR peak table for terpolymer.......................................................103
2.2. Raman peak table............................................................................104
3.10. FTIR Peak Table...........................................................................130
Bibliography


30. Catherine, H.; Skinner, W.; Osbaldiston, G. W.; Wilner, A. N. 


95. Li, M.; Mann, S. Langmuir, 2000, 16, 7088.


Declaration

I hereby declare that, this Ph.D. thesis is original and is the result of my work in the Department of Experimental Physics, University of Ulm, Germany under the supervision of Prof. Dr. Othmar Marti. I further declare that this work has not submitted anywhere else for the requirement of any other degree.

Ulm,
June 2005. Ranjith Krishna Pai
Acknowledgement

I chose a career in polymer chemistry when I was an undergraduate student, but at that time I did not imagine that I would study in a foreign country for my Ph.D. research. I even boasted “if I can learn polymer science in India, why do I have to go to a foreign country!” Now I am in my office and have finished writing my Ph.D. thesis, at the University of Ulm, Germany - the birth place of Albert Einstein. Life is full of unexpected things. Sometimes, however living in foreign countries is tougher than one can imagine. You have to use the foreign language and deal with people whose customs you do not understand. And, you do not have your old friends or family around you.

But many people survive and I did and even enjoyed life in Germany very much. It is because of the people around me, who helped me in many ways. Without their help, I would not have been able to finish my work. I would like to show my deepest appreciation for all of you here in Ulm. To be honest, I do not like repeating “thanks” for many people. I think it can shrink the meaning of “thanks”. But I can not find any better way, so please allow me to say “thanks” many times. If you can not find your name here, it does not mean I do not appreciate you, simply I do not have enough space here (if you know me well, you know there are too many people I owe something).

First and foremost, I want to thank Prof. Dr. Othmar Marti for being my thesis advisor and providing excellent working conditions. Prof. Marti taught me many aspects of AFM, kindly and with
incredible patience. I truly appreciate that he took me to several interesting places to show me nature and landscapes.

I would like to thank Prof. Dr. Katharina Landfester (Organic and Macromolecular Chemistry) for her immense guidance for synthesizing calcium carbonate by the miniemulsion method. She was always there to proof-read my chapters and asked me good questions to help me think through my problems.

Special thanks go to Dr. Bernd Heise who gave me untiring help during difficult moments, for support and helpful discussions and for the English-German translation of the abstract.

Thanks to Prof. Dr. Dirk Volkmer (Inorganic Chemistry II) for support and helpful discussions throughout this work. When I think in retrospect, why I came to Ulm and why things went so well, it is obvious that things are linked more or less directly to my former supervisors Dr. S. Sivaram and Dr. R. A. Kulkarni. I am grateful for the good research experiences and the lessons I learned from their group in the National Chemical Laboratory (NCL) in India. Special thanks to Dr. R. A. Kulkarni for giving me the guidance to synthesize acrylamide based water-soluble polymers and permission to use these polymers for my Ph.D. work.

Special thanks go to my collaborators, Dr. Andreas Ziegler (Central Facility for Electron Microscopy), Dr. Sabine Hild, Prof. Dr. Matthias Epple and Alexander Becker (Institute of Inorganic Chemistry, University of Duisburg-Essen) and Prof. Dr. Stephen Mann (School of Chemistry, University of Bristol, UK) who are most responsible for helping me to complete the writing of this dissertation as well as the challenging research that lays behind it.
Dr. Andreas Ziegler has been a friend and mentor. He had confidence in me when I doubted myself, and helped bring out good ideas from within me. Without his encouragement and constant guidance, I could not have finished this dissertation. He was always there to meet and talk about my ideas, to proof-read, mark up my papers & chapters and asked me good questions to help me to think through my problems.

I wish to express my warm and sincere thanks to Prof. Dr. Martin Pietralla and Dr. Gerd-Ingo Asbach for their support and helpful discussions throughout this work. I owe most sincere gratitude to Saju Pillai, who gave me untiring help during my difficult moments. His ideals and concepts have had a remarkable influence on my entire career in the field of research.

I thank my colleagues in the department of Experimental Physics group. They were all nice and kind to me and provided many scientific and other small discussions. Especially my “Deutsch” colleagues who taught me how to be Deutsch in this country.

Lots of thanks to Nicola Maghelli for sharing his experience in the endeavor of dissertation writing. With Alexander Schmatulla, Raghavan Chinnadurai, and Devi Rajan I enjoyed a nice atmosphere and good scientific and non-scientific discussions. Thanks to Martin Hinz for helping me to operate the DI. I would like to give a very special thank to Mrs. Tamara Stadter for her administrative help and thank to Monika Pahwa for English language tips.

The Scholarship from the graduate college 328 “Molecular Organization and Dynamics at Interfaces” University of Ulm, is gratefully acknowledged.
Last, but not least, I thank my parents for educating me with aspects from both arts and sciences, for unconditional loving support and encouragement to pursue my interest, even when the interest went beyond boundaries during all these years. And thanks to my brother Rajesh K Pai and sister Ranjini K Pai for reminding me that my research should always be useful and serve good purposes for all of humankind.

Ranjith Krishna Pai
Publications and manuscripts in preparation


Posters and Conferences


2. Presented poster in the Common Workshop of GRK 328 “Molecular Organisation and Dynamics at Interfaces” and SFB 569, 12th – 14th October 2003 in Heinrich-Fabri-Institut, Blaueuren, Germany.


5. Presented poster in the Common Workshop of GRK 328 “Molecular Organisation and Dynamics at Interfaces” and SFB 569, 26th – 27th November 2004 in University of Ulm, Germany.

6. Participation at the Advanced Biomaterial course & undergone examination and secured grade 2, conducted by Pof. Dr. L. Claes, University of Ulm.