Biomineralization and Biomimetic Materials Chemistry: Exploring the Influence of Crystal Growth Modifier

Submitted by

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Synopsis

SYNOPSIS

The thesis entitled, “Biomineralization and Biomimetic Materials Chemistry: Exploring the Influence of Crystal Growth Modifier” is divided into four chapters.

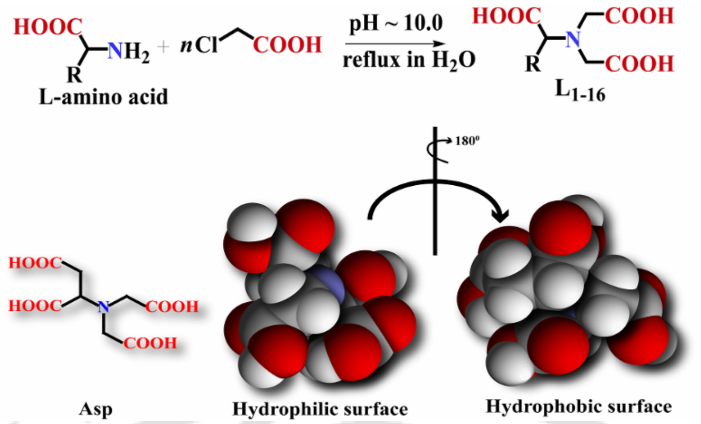
Chapter-1 gives a brief introduction regarding background as well as literature survey of Biomineralization. It describes how in nature, hard tissues of organism such as bone, teeth and mollusk shells *etc.* forms and their characteristics. It also describes the literature survey of *in vitro* biomineralization in presence of low as well as high molecular weight matrix.

The various bioinorganic materials present in living systems are biominerals. *e.g*, Hydroxyapatite [$Ca_5(PO_4)_3(OH)$], Calcite ($CaCO_3$) *etc.* They are formed by all the living organisms starting from bacteria to higher plants and animals. It has shapes and size which are frequently different from analogous inorganic crystals. They are organized hierarchallly and ordered at many length scales. Biominerals have very low solubility in the body fluid. Low solubility product of Ca salt is the main reason why it is the most abundantly found element in the naturally occurring biomineral. They are thermodynamically stable than the analogous inorganic crystals due to the consideration of the ionic size, charge, packing, and their effect on hydration and lattice energies.

Biomineralization is the process of mineralization in living organisms or *in vivo* in an organized manner under the control of acidic organic macromolecules (protein, amylose, etc.) excreted by the specific positions of living organisms, and the hallmark of this process is that the nucleation of the inorganic phase is strictly controlled by the organic phase. The materials can be deposited intra or extra cellular space and are intimately connected to cellular metabolic processes.

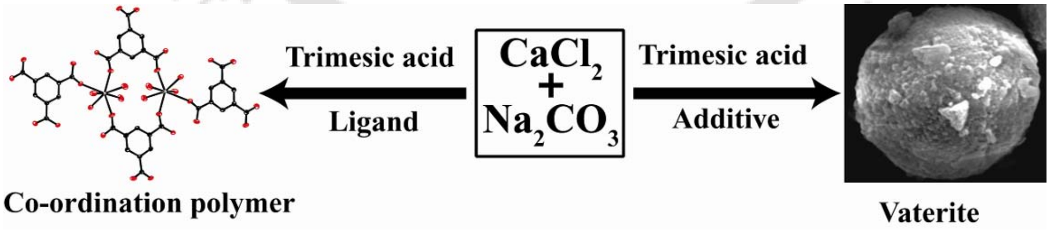
Chapter-2 discusses the role of low-molecular-weight growth modifier in biomineralization. This chapter is subdivided into two parts. First part discusses about biomineralization in presence of low-molecular-weight poly-carboxylate derived from amino acids. Second part describes about the influence of organic and inorganic additives in $CaCO_3$ polymorph synthesis.

For last two decades, extensive studies have been done on the biomineralization of Ca. It has been established that the biominerals grow on the organic template containing O and N donor atoms. Group II metals prefer hard O donor ligands according to HSAB principle. A study of $CaCO_3$ crystal binding motif of naturally occurring biominerals reveal the fact that they are grown on proteins containing a special kind of sequence that mainly contains Asp and Glu residues. Depending on the amino acids present at the nucleation center biominerals grow with different morphology that performs various physiological roles. The synthesis of poly-carboxylate ligands derived from naturally occurring α-amino acids reacting with chloro acetic acid at basic pH following Scheme 1.



Scheme 1. Synthesis of poly-carboxylate ligands and its space fill model.

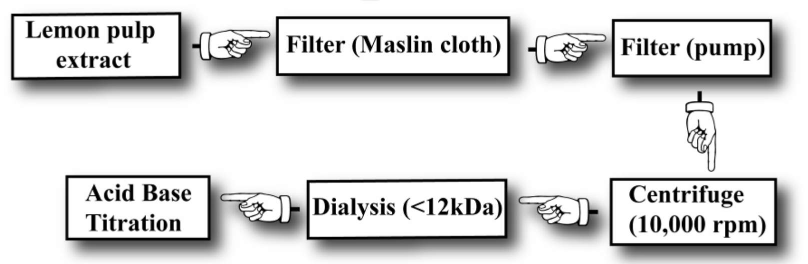
The polymorph synthesis of $CaCO_3$ in presence of various organic as well as inorganic additives in different solvents is presented in the second part. The effect of additives has been discussed mechanistically. Vaterite is the major polymorph in non-aqueous solvents irrespective of additives. Coordinating organic additives form extended 3D co-coordination polymers when used stochiometrically. These metal complexes form exclusively porous calcite on thermal decomposition. To establish the thermodynamic stability of different polymorph of $CaCO_3$, temperature dependent experiments were carried out in detail (Scheme 2). Calcite is the major polymorph at two extreme temperatures (-190^{o}C and 189^{o}C).



Scheme 2. Additive control polymorph synthesis of $CaCO_3$.

Chapter 3 concerns with the biogenic mineral growth modification in presence of naturally occurring high-molecular-weight matrix. This chapter is divided into three parts. First part discusses the role of naturally occurring high-molecular-weight acidic proteins from lemon extract as a growth modifier of calcium and barium minerals. Second part describes the role of bacterial proteins as a high-molecular-weight organic matrix and the third part deals with biomimetic modulation of crystal morphology using gel diffusion techniques.

Crystalline superstructures of Calcium and Barium minerals were synthesized that form micron-sized building blocks in the presence of high-molecular-weight proteins extracted from lemon as shown in the scheme 3, which act as an organic growth modifier. Spherulitic growth of crystalline calcite on the expense of metastable amorphous calcium carbonate (ACC) has been observed (Figuire 1).



Scheme 3. Extraction of high-molecular-weight acidic proteins from lemon.

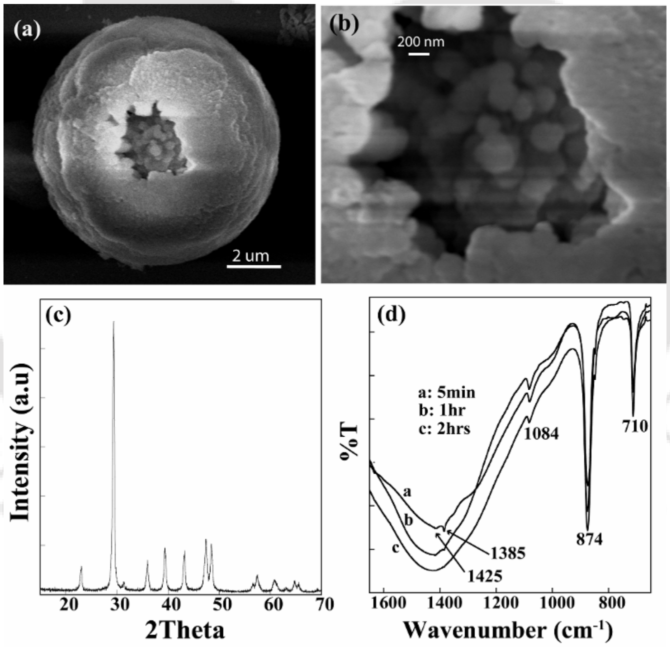
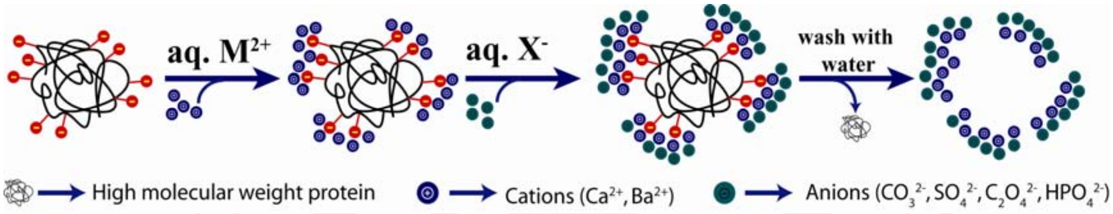


Figure 1. (a) SEM micrographs of hollow spherulitic Calcite, (b) Calcite nano-spherulites, (c) PXRD pattern of Calcite crystals and (d) Time dependent FT-IR analysis with characteristics humps at 1425 & 1386 cm^{-1}.

The role of three different lactic acid bacteria (LAB) *viz*: *Lactobacillus plantarum* MTCC 1325, *Lactobacillus acidophilus* MTCC 4495 and *Pediococcus acidilactici* CFR K7 in the formation of crystalline superstructures of calcium and barium minerals that forms micron-sized building blocks has been discussed in this second part of the chapter. The influence of these bacterial extracts on the crystallization behavior was investigated in details to test the basic coordination behavior of the acidic protein. The significant role of native form of high-molecular-weight bacterial protein extracts in the generation of nucleation centers for crystal growth is also established. A model for the formation of organic matrix-cation complex and the subsequent events leading to crystal growth modification is proposed (Scheme 4). Reproducibly formations of highly pure crystalline minerals phase are highlighted here.



Scheme 4. Proposed mechanism of template action of protein extract.

Gel-diffusion-precipitation methodology is unique. This effective method is capable of characterizing the effects of matrix molecules on mineralization while only using very small quantities of material. In this part we have discussed the versatility of agar-agar and agarose gel in morphosynthesis of different calcium and barium minerals. We have shown the formation of different nano to micron-scale architectures of calcium and barium minerals in presence of these hydrogels. They are formed by well known gel diffusion technique when doped with soluble calcium/barium salts and anions are allowed to diffuse through pores (Figure 2).

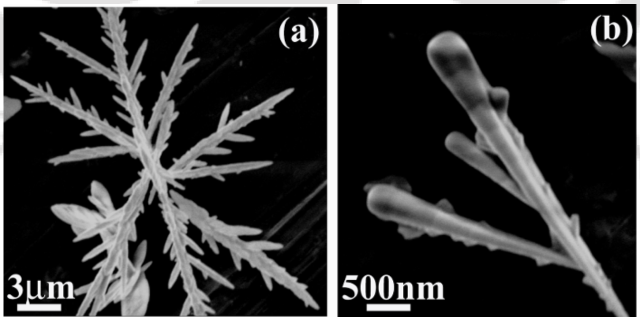
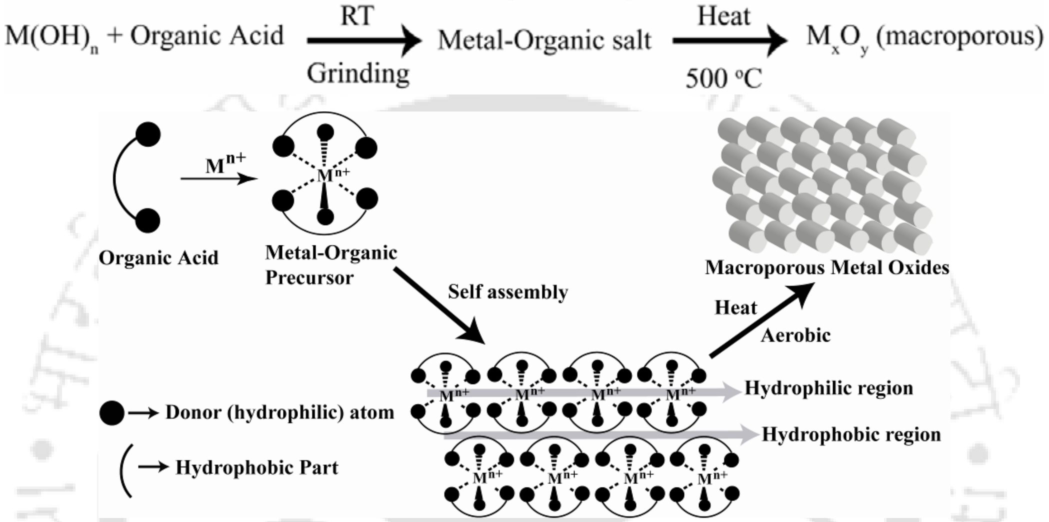


Figure 2. Formation of Barite needles in presence of (a) agar-agar and (b) agarose gel.

Chapter 4 deals with precursor synthesis of inorganic minerals and their characterization. This chapter is subdivided into three parts. First part discusses the synthesis, characterization and application of metal oxides by precursor synthesis method. Second part discusses synthesis, characterization of metal ferrites and the final parts deals with magnetite mineralization and its surface modification.

Precursor synthesis is one of the well established solid-state methods to prepare inorganic mineral. Crystalline precursor complexes transform to other known crystalline mineral phases at higher temperatures *via* calcination-reconstruction method. Among the various inorganic materials, metal oxides have attracted increasing technological and industrial interest. We have carried out synthesis of few metal oxides by precursor synthesis method following Scheme 5. Such synthetic methodology is easy, quantitative and reproducible. All organic precursor salts and thermally decomposed products were characterized in detail (Figure 3).



Scheme 5. Synthesis of metal oxides by thermolysis of Metal-organic precursor salts.

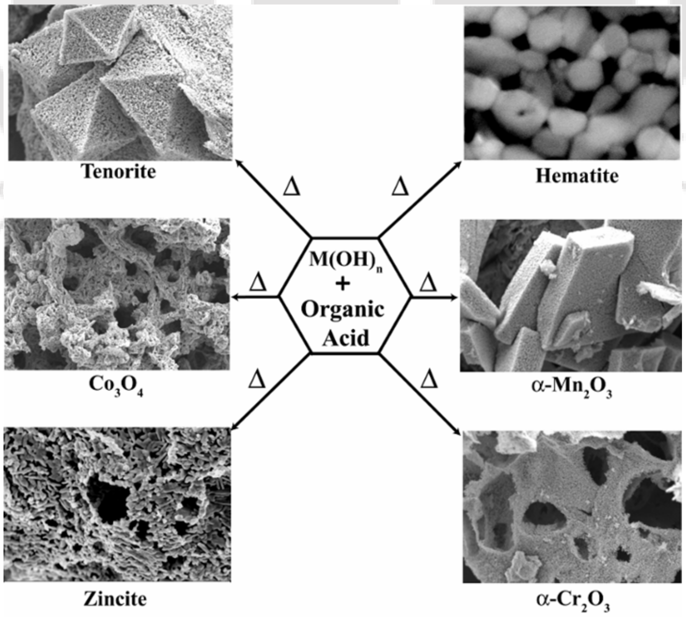
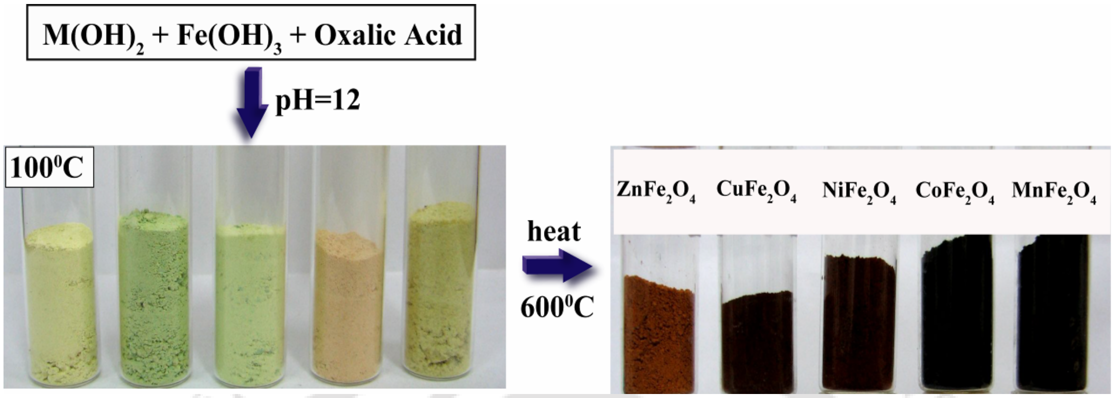


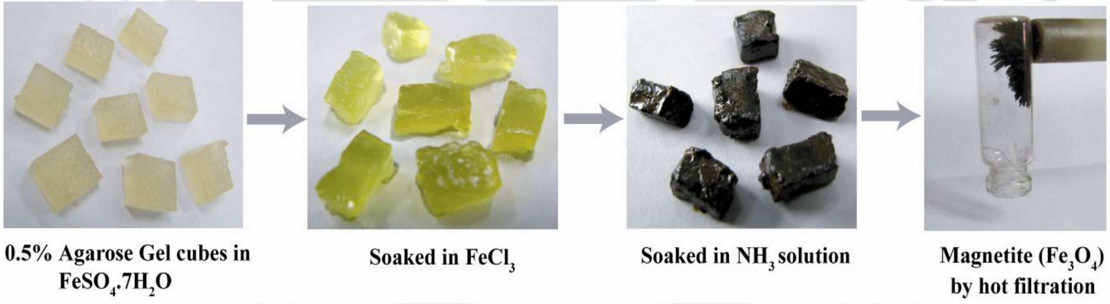
Figure 3. Macroporous metal oxides obtained by thermal decomposition of precursor complex.

Spinel of the type $M^{2+}M_2^{3+}O_4$, when M^{3+} = Fe, the resulting spinel ferrites having a general chemical composition of MFe_2O_4 (M = Mn, Co, Ni, Cu, Zn) have been widely applied as magnetic materials in various biomedical applications. We have carried out synthesis of soft ferrites by precursor synthesis method following Scheme 6.



Scheme 6. Synthesis of metal ferrites by thermolysis of M(II)-organic precursor salts.

Magnetite (Fe_3O_4) is a natural magnet and is a member of the spinel group. Magnetite biomineralization is a universal phenomena which is evident in a number of microorganisms *e.g.* magnetotactic bacteria under ambient conditions. We report here the formation and growth modification of magnetite micro crystalline minerals in Agarose gel (Scheme 7).



Scheme 7. Magnetite mineralization in Agarose Gel.

We have carried out surface modification of these magnetite particles by Trimesic acid (TMA) and stearic acid. Surface modification results in the complete change in their adsorption behavior, which has various practical applications such as protein adsorption, separation etc. Stearic acid modification generates a hydrophobic surface upon coating on magnetite particles whereas trimesic acid modification generates an improved hydrophilic surface. Change of the water adsorption behavior of the stearic acid coated magnetite particles has been shown in Figure 4.

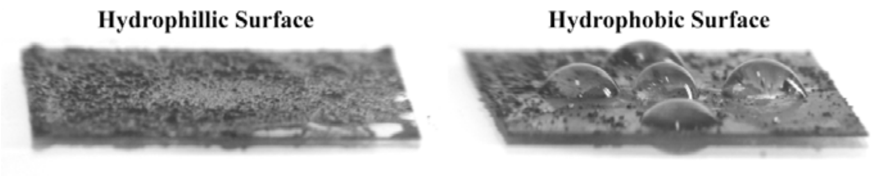
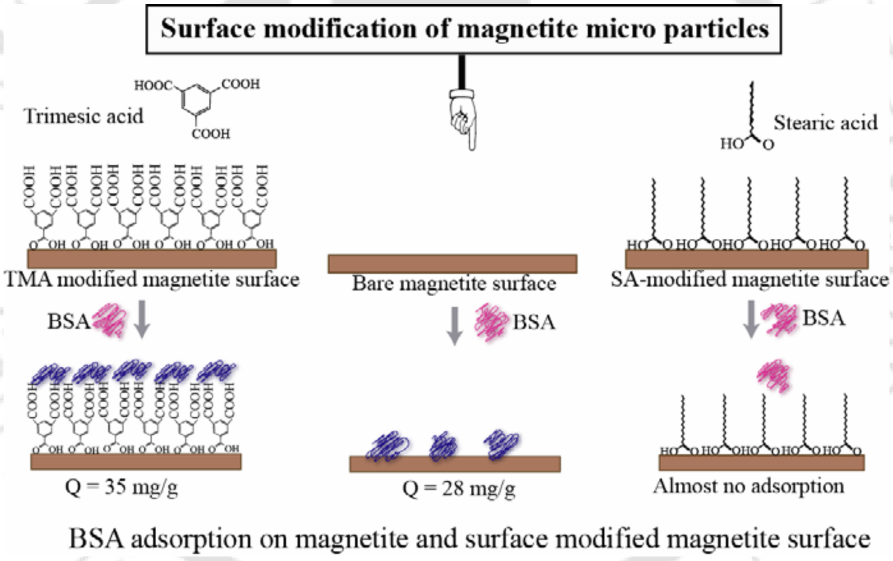


Figure 4. Surface modification of magnetite particle with stearic acid.

We have studied the protein interaction phenomenon with bare magnetite particles and the changes in adsorption features upon surface modification. Bovine serum albumin (BSA) was taken as a model study. Detail conformational features of BSA are known therefore it would be easier to analyze the conformational change of BSA at the interface upon adsorption (Pink-native BSA, Blue-changed BSA). Equilibrium adsorption capacity and protein conformation at the interface was studied with these as-prepared and surface modified particles. Fluorescence labeled magnetite particles has been synthesized by adsorbing Fluorescein onto the surface as shown in Figure 5.



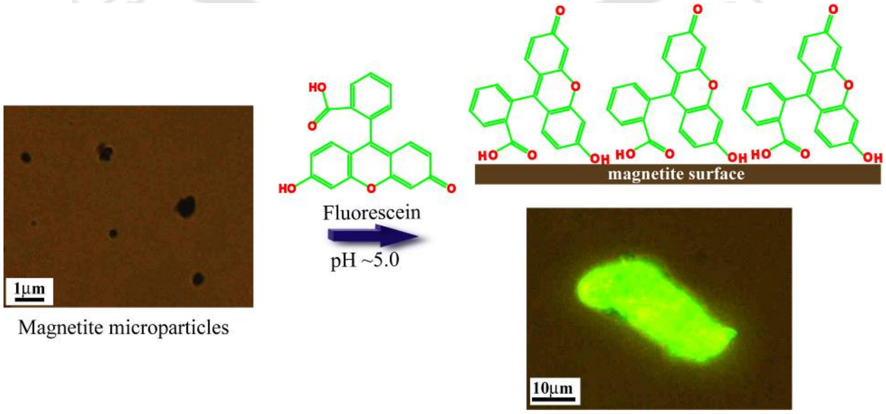


Figure 5. Adsorption of fluorescein by magnetite micro particles.