Effect of additives on bio-inspired mineralization of $CaCO_3$ and application of its composites

A Dissertation

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The thesis entitled **“Effect of additives on bio-inspired mineralization of $CaCO_3$ and application of its composites”** is categorized into eight chapters based on the results obtained by experimental works performed during the research period.

Chapter 1: Introduction

Biomineralization is a fundamental life process that leads to the formation of mass and structure in almost all life forms. This biogenic process leads to the formation of hierarchically structured organic-inorganic hybrid composites, popularly termed biominerals, which are widely prevalent among almost all living organisms.[1–3] The organisms exploit the properties of these materials, such as mechanical, optical, magnetic, etc., for a variety of purposes.[4,5] The organisms undergo evolution in the biomineral structures based on their requirements and to adapt to specific environmental challenges. It is fascinating to observe the ability possessed by the organisms to optimize the shape, size, structure, orientation, texture, and overall morphology of the biominerals. The scientists have involved themselves in understanding this biological phenomenon and more so have engulfed themselves in understanding the mystery behind this art. Till today, decades since the onset of the preliminary studies, a fairly large number of questions related to biominerals still remain unexplored. The scientific efforts in the field of materials science, clubbed together with the understanding of chemistry, have enabled scientists to mimic the design and synthesize various biogenic materials, of which the biominerals commandingly stand out as one of them.[6–8]

The study of biomineralization is a multidisciplinary prospect that engages researchers from biology, chemistry, materials science, geology, and beyond.[9] The key role played by chemistry in biomineralization research can broadly be divided into three main areas; in studying the chemical composition, biochemistry, and crystallography of the materials, in designing *in vitro* model systems to understand the interactions between the organic and the inorganic phases to understand the actual mechanism in biological systems and in developing new synthetic techniques by mimicking the biological systems to generate organic-inorganic composites with enhanced mechanical properties.

Among others, the factor that stands out is the ability of $CaCO_3$ to exhibit polymorphism. For over a century, $CaCO_3$ has been known to exist in three main anhydrous crystalline forms; calcite, aragonite, and vaterite, two hydrated crystalline forms; calcium carbonate monohydrate (monohydrocalcite) ($CaCO_3.1H_2O$) and calcium carbonate hexahydrate (ikaite) ($CaCO_3.6H_2O$), and also in the amorphous state as amorphous calcium carbonate (ACC).[10]

The crystal morphology, polymorphism, and materials properties of the biominerals can be controlled by the introduction of templates and additives. The introduction of a template to influence the nucleation step during $CaCO_3$ crystallization is fundamentally referred to as heterogeneous nucleation and the effect induced by it is known as the templating effect. Since its discovery in 1965, the presence and also the distribution of intracrystalline molecules in various marine organisms,[11] the researchers working on biomimetic materials based on biominerals have moved a long way in their study of the incorporation of species within the composite. These incorporated species are known as additives and they play a crucial role in determining the properties of the composite.

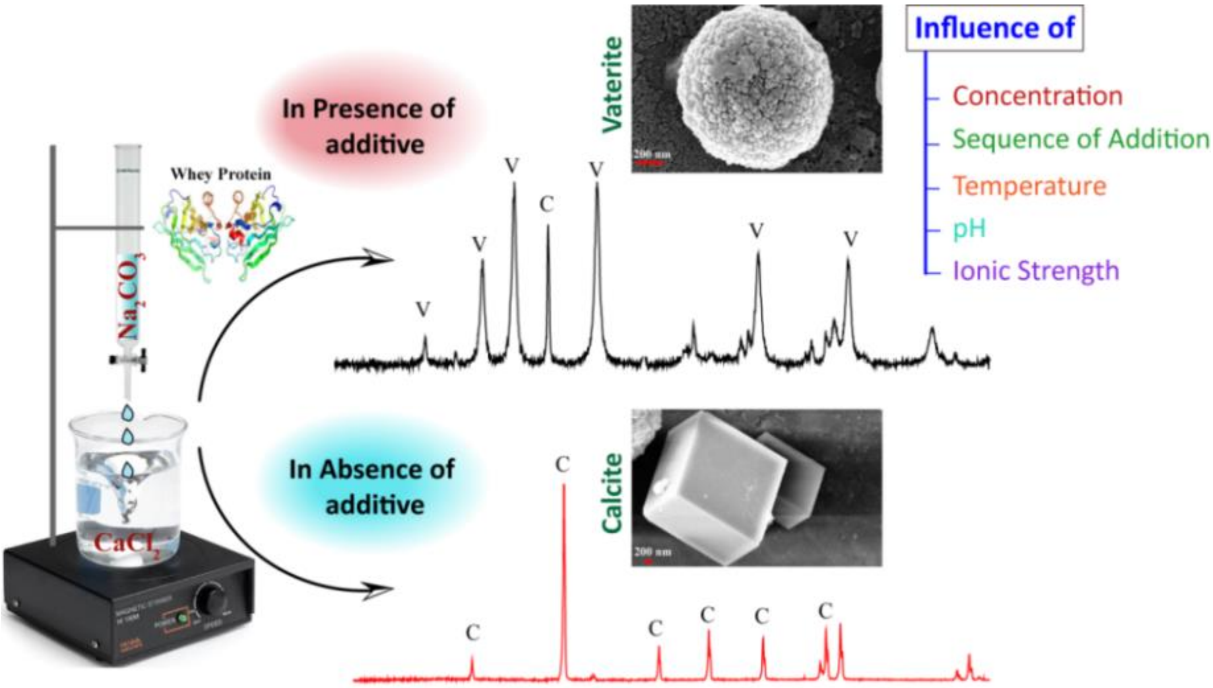
$CaCO_3$ is a very important material for industries. The biomimetic method of its synthesis can give us any of the polymorphs, ACC, calcite, vaterite and aragonite, or a mixture and accordingly, various morphologies can be synthesized of nano to microsized particles. These *in vitro* biomineralized $CaCO_3$ have a wide range of applications, including as drug delivery vehicles, bone grafting, protein immobilization, catalysis, biosensors, CO_2 capture and release and water treatment.

Chapter 2: Experimental Methods and Characterization

This chapter provides comprehensive information about the materials and different methodologies adopted for the synthesis and characterization of the biominerals and their composites, followed by their subsequent applications. Specification of analytical instruments used along with different experimental conditions adopted in the study of the materials and their applications have been elaborately discussed.

Chapter 3: Whey protein directed *in vitro* biomineralization of vaterite: A study on the influence of external parameters on $CaCO_3$ phase transformation (*Colloid Interfac. Sci. Comm.*, 2020, **36**, 100255)

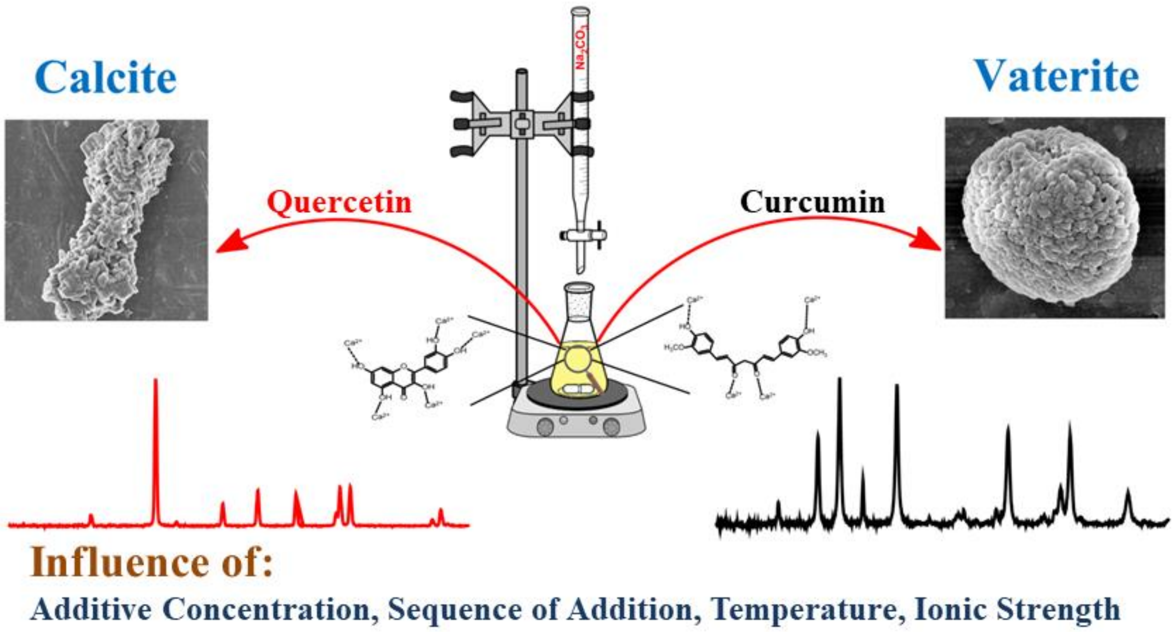
This chapter demonstrates the role of a complex biopolymer, the organic biomolecule whey protein as an additive *in vitro* biomineralization of $CaCO_3$. Whey protein is composed of five major components, α-lactalbumin, β-lactoglobulin, glycomacropeptide, serum albumin, and immunoglobulins. As a complex biopolymer, whey protein exhibits a random conformation in solution and in its role in the process of *in vitro* biomineralization of $CaCO_3$; its adsorption on the initial nanocrystals of $CaCO_3$ via weak interactions influenced the kinetic crystallization pathway, hence selectively forming the metastable vaterite phase rather than the most stable calcite phase. The study was extended to understand the influence of the change in parameters such as the concentration of the additive, sequence of addition, temperature, pH and ionic strength of the solution on phase transformation and to justify the role of whey protein in assisting the formation of the metastable vaterite particles.



Scheme 1: A pictorial illustration of the research work covered in **Chapter 3**.

Chapter 4: Role of curcumin and quercetin as additives in the *in vitro* biomineralization of $CaCO_3$: A comparative study on phase modulation (*Ceram. Int.*, 2021, **47**, 12334-12341**)**

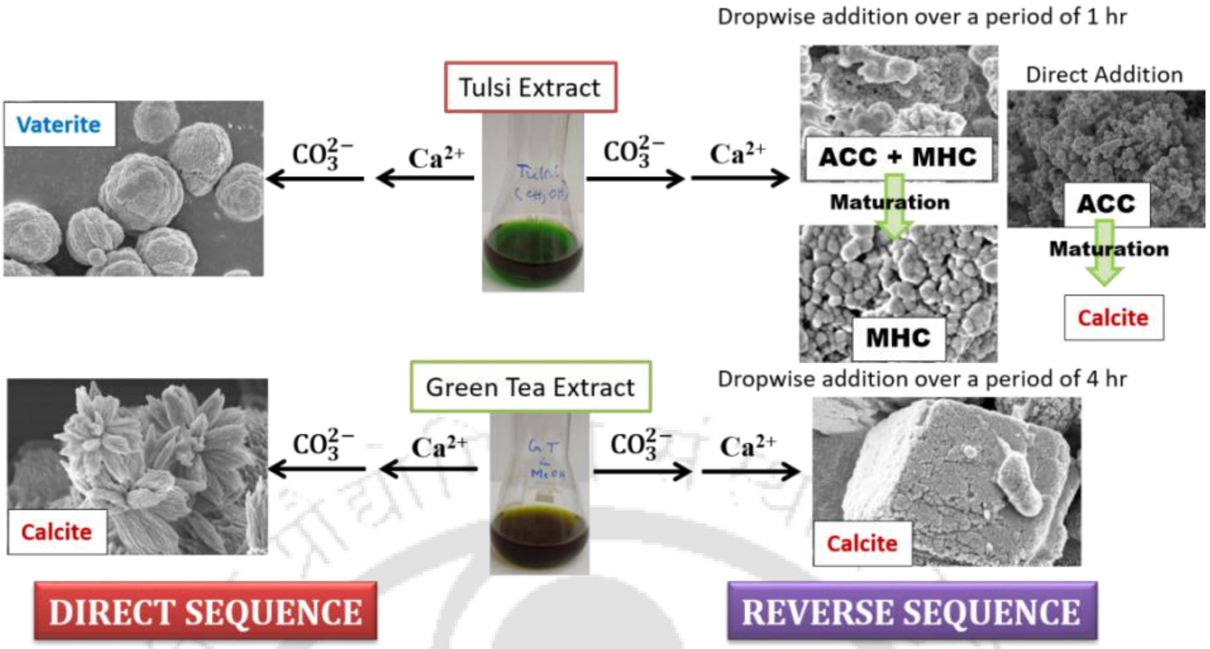
The use of low molecular weight organic molecules as additives in the *in vitro* biomineralization of $CaCO_3$ was discussed in this chapter. A comparative mode of study was performed between two polyphenolic additives, curcumin and quercetin; both of them being bio-friendly with numerous health benefits. The additive curcumin was able to stabilize the metastable vaterite phase and with quercetin, an interesting observation was made in terms of the morphology of the calcite particles formed. A dumbbell-shaped morphology, different from the usual rhombohedral morphology of the calcite crystals, was obtained. Additionally, the maturation of the $CaCO_3$ crystals in solution with time was studied to observe any changes in phase and morphology. Also, extended studies were performed to investigate the influence of the change in parameters such as; template concentration, the sequence of addition, temperature and ionic strength on the phase, morphology and size of the particles in the presence of both the additives.



Scheme 2: A pictorial illustration of the research work covered in **Chapter 4**.

Chapter 5: Tuning of $CaCO_3$ phase and morphology directed by plant-based *in vitro* model systems: A thorough insight into the formation of monohydrocalcite (*New J. Chem.*, 2021, **45**, 18337–18348**)**

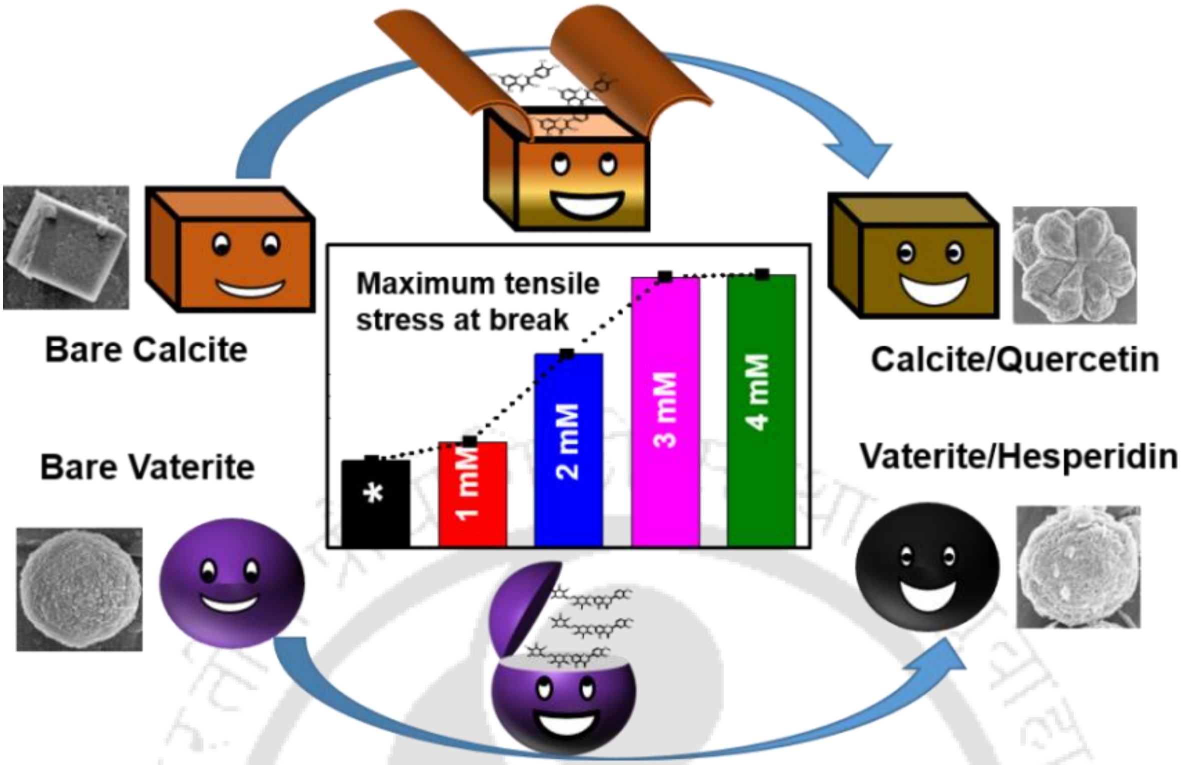
In this chapter, an *in vitro* study on the modulation of phases and polymorphism of $CaCO_3$ utilizing some naturally available and biological friendly additives was performed. The study was based on Tulsi and Green tea extracts in methanol and water. In the first part of the study, metastable vaterite particles and calcite particles with highly captivating and elegant floral morphology viz. rose-like and daffodil-like, were successfully synthesized. The second part involved reversing the sequence of addition of the Ca^{2+} and CO_3^{2-} ions where we could isolate the highly unstable amorphous calcium carbonate and one of the rare hydrated phases of $CaCO_3$, the monohydrocalcite phase. The formation of monohydrocalcite was obtained by controlling the addition of the Ca^{2+} ions, and the subsequent transformation of the monohydrocalcite particles to the thermodynamically stable calcite phase at high temperatures was studied. In short, the *in vitro* transformation of $CaCO_3$ from the unstable amorphous precursor to the stable calcite phase via the hydrated monohydrocalcite phase was reported with experimental evidences including crystallographic studies and well defined morphologies.



Scheme 3: A pictorial illustration of the research work covered in **Chapter 5**.

Chapter 6: Incorporation of flavonoids into $CaCO_3$ and their influence on the phase, morphology and mechanical strength of the composites: A bio-inspired approach (*Colloids Surf. A: Physicochem. Eng. Asp.*, 2022, **642**, 128720**)**

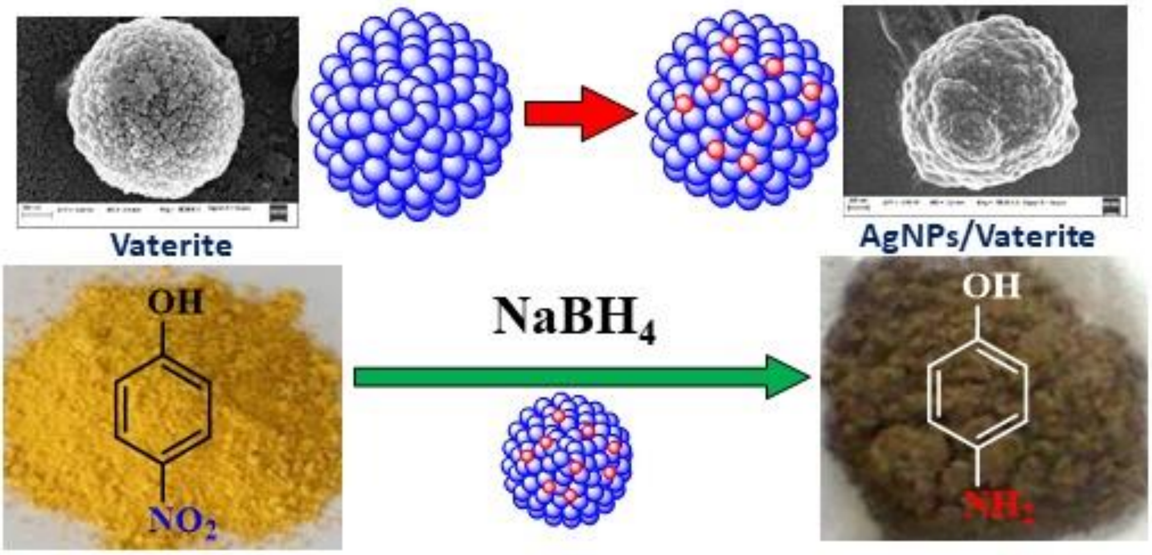
Biominerals are composite materials that exhibit remarkable mechanical strength and properties and the organic molecules associated with the inorganic crystalline phase stand out as one of the most important reasons for this phenomenon. The work discussed in this chapter involves *in vitro* synthesized biominerals based on the flavonoids quercetin and hesperidin. In the first part of this study, the influence of these organic molecules on the resulting $CaCO_3$ composites in terms of phase and morphology is illustrated. Subsequently, in the second part, it is demonstrated how by tuning the concentration of the incorporated organic phase, constituted by the flavonoid molecules, the mechanical toughness of the composite could be varied. The interaction between the organic and inorganic phases and their influence on the mechanical properties of the composite for both calcite and metastable vaterite is described. The mechanical strength of the composites was evaluated post their doping into gelatin films. The maximum tensile stress attained by the $CaCO_3$ incorporated with quercetin and hesperidin was 2728 kPa and 3152 kPa, respectively, in contrast to the low value of 1140 kPa, which was observed for bare $CaCO_3$.



Scheme 4: A pictorial illustration of the research work covered in **Chapter 6**.

Chapter 7a: *In vitro* biomineralized vaterite as a solid-support for silver nanoparticles: A highly efficient catalyst for the reduction of 4-Nitrophenol (*Mol. Catal.*, 2021, **504**, 111433)

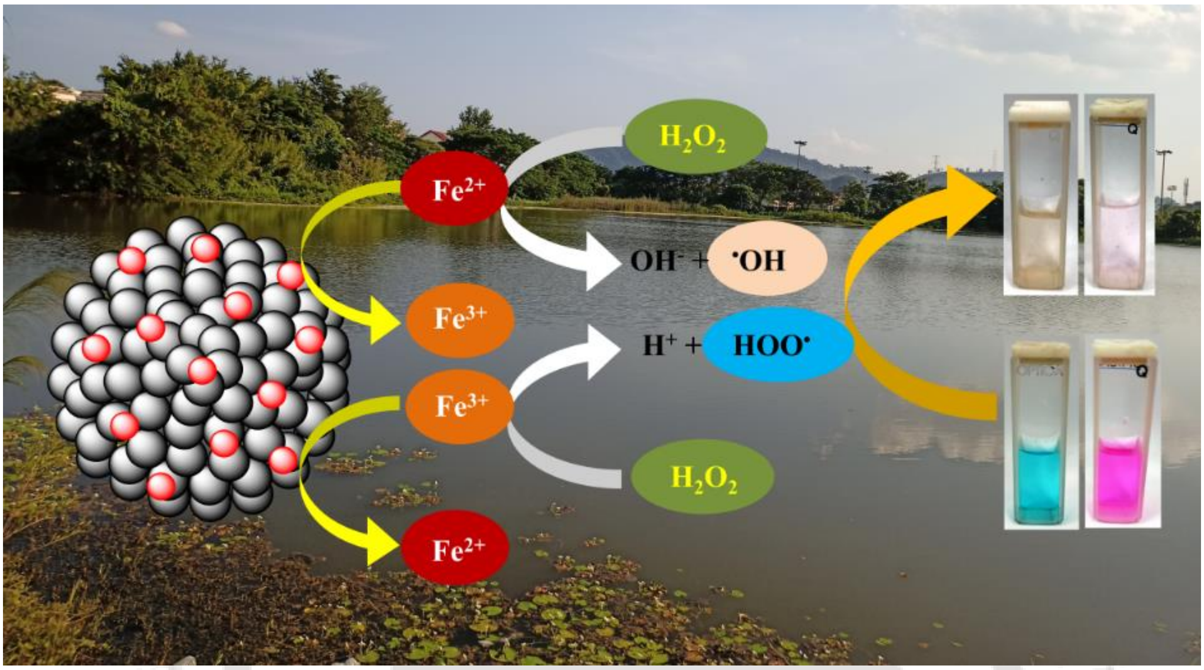
In this work, the spherical morphology and porous surface texture of biomineralized vaterite were utilized by subjecting it as an inert solid support for silver nanoparticles. The resulting composite material was used as a catalyst for the reduction of 4-Nitrophenol. The methods assigned were eco-friendly and one of the stand out features was the very even and dense distribution of the silver nanoparticles with sufficient spacing between the neighbouring particles such that the question of agglomeration between particles could be neglected. The majority of the silver nanoparticles embedded were of ultrafine size, with an average diameter of 3.5 nm, which can be attributed to the porous surface texture of vaterite. The composite catalyst showed excellent catalytic activity towards the reduction and remained unaltered for at least five consecutive cycles. The high rate constant of 2.2×10^{-2} s^{-1} and an impressive turnover frequency of 2.36×10^{-2} mmol mg^{-1} s^{-1} are the highlights of this nanoparticle-based catalyst. Additionally, the effectiveness of this catalyst towards the reduction of 4-Nitrophenol were also studied in water from natural sources; tap water and lake water. The results attained were highly impressive, suggesting that the material promises to be highly efficient as a potential catalyst for the industrial treatment of wastewater and can be deployed for large-scale purposes.



Scheme 5: A pictorial illustration of the research work covered in **Chapter 7a**.

Chapter 7b: Vaterite and calcite based magnetite composites: A comparative study on the catalytic performance during spontaneous Fenton-like dye degradation (*J. Environ. Chem. Eng.*, 2022, **10**, 107558**)**

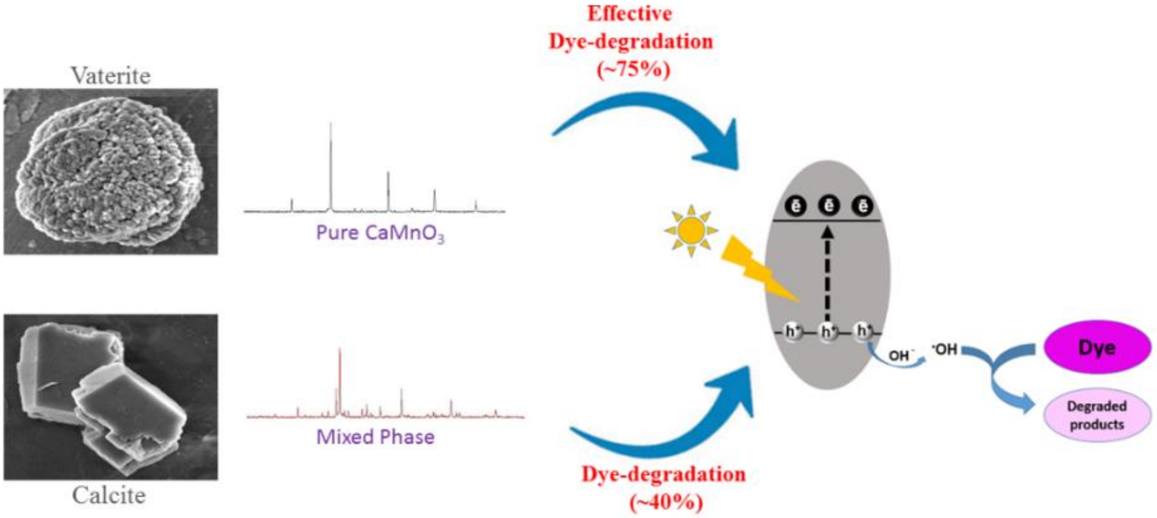
This chapter is based on an advanced oxidation process (AOP), the Fenton-like degradation of dyes. AOPs have been adopted to address the increasing threat of wastewater discharge from industries. In this work, an attempt was made to improve the performance of magnetite (Fe_3O_4) nanoparticles when subjected as catalysts in Fenton-like dye-degradation. *In vitro* biomineralized vaterite was used as solid support for Fe_3O_4 nanoparticles, and the resulting composite was utilized as a heterogeneous catalyst to perform the degradation of potentially toxic organic dyes, Methylene Blue (MB) and Rhodamine B (RhB). With bare Fe_3O_4, degradation of 60% and 58% of MB and RhB was observed in two hours, whereas, with the vaterite-supported Fe_3O_4, almost complete degradation, 96%, of MB and 86% degradation of RhB was achieved. As a comparison, the commercially available form of $CaCO_3$, the calcite phase, was also used as a support for Fe_3O_4 and the composite, when used as a catalyst, resulting in 81% and 73% degradation of MB and RhB. The comparative studies revealed that the use of solid supports had improved the catalytic activity of the material. The biomineralized vaterite microspheres behaved as better support, and therefore, high rate constants of 3.15×10^{-2} min^{-1} and 1.82×10^{-2} min^{-1}, respectively, were achieved for the degradation reactions of MB and RhB performed using the catalysts based on them. Further, the magnetic nature of the catalyst made it a great prospect for reusability. Experimentally we observed that the catalysts retained their complete efficiency even after five consecutive cycles. Also, the versatility of the catalyst was proved by its ability to perform in natural water sources; tap water, lake water and river water.



Scheme 6: A pictorial illustration of the research work covered in **Chapter 7b**.

Chapter 8: *In vitro* biomineralized vaterite derived efficient solid-state synthesis of pure $CaMnO_3$ perovskite as a useful photocatalyst (_CrystEngComm._, 2021, **23**, 4050-4058**)**

The perovskite materials have attracted immense popularity in recent years due to their role in energy-based applications. Calcium manganese oxides are analogous to the systems that perform water-splitting in the natural photosynthesis process. Herein, we discuss a convenient method to synthesize the perovskite $CaMnO_3$. Biomineralized vaterite was used as the calcium source to synthesize pure $CaMnO_3$ efficiently. In contrast, commercially available $CaCO_3$ comprising of the calcite phase led to the formation of a mixture of calcium manganese oxides. The bandgap energy of pure $CaMnO_3$ was found out to be 1.21 eV. This low value suggested that the material would easily absorb visible light, rendering it highly suitable to be used as a photocatalyst. Hence, it was subjected as a photocatalyst to catalyze the photolytic degradation of the toxic dyes viz. Rhodamine 6G, Rhodamine B, Methylene blue and Methyl orange. The photocatalytic degradation of Rhodamine 6G was studied in detail, where 72% of dye degradation was observed within 2 hrs in the presence of pure $CaMnO_3$ in contrast to 46% in the presence of the mixed-phase calcium manganese oxide. The mechanistic pathway of degradation was also studied, from which we could infer that the photo-generated ${}^{\bullet}OH$ and h^+ were the species responsible for the degradation of Rh 6G. To validate the effectiveness of the catalyst towards wastewater treatment, the degradation experiments were performed in lake water and river water, and the results obtained were impressive.



Scheme 7: A pictorial illustration of the research work covered in **Chapter 8**.

Conclusion and future plans and perspectives

To summarise, all the aspects of biomineralization broadly covered by a chemistry have been addressed in the experimental works, leading to the preparation of the thesis. The characterization of the crystallography and composition of the materials to study the phase and morphology of the biominerals; designing *in vitro* model systems to understand biomineralization where both small and large molecules, as well as plant extracts, were used; and in the development of new methods to improve the material properties, the incorporation of flavonoids to improve the mechanical strength was performed; also the biomineralized materials were subjected as solid supports for metal nanoparticles and converted to perovskite materials to perform wastewater treatment related applications. Further, the utilization of the *in vitro* biomineralized $CaCO_3$ particles in biological applications such as drug delivery and a similar approach with another calcium-based biomineral hydroxyapatite are in the reckoning. The results attained through the course of work show how biologically useful molecules and plant extracts can participate in Ca-based biomineralization and hence, generating scope for their utilization in the broader picture concerning these materials, such as in applications involving drug delivery vehicles, bone grafting, etc. In addition to the applications performed in the experiments leading to this thesis, the numerous polymorphs of $CaCO_3$ synthesized here can be utilized in other medical and environmental applications.

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