Droplet-Based Microfluidics for the Study of CaCO₃ Crystallisation

Alexandra Yashina

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A thesis submitted in partial fulfillment of the requirement for the degree of
Doctor of Philosophy
Declaration of Originality

Except where specific reference is made, the material contained in this thesis is the result of my own work and has not been submitted in any form for another degree or diploma at this or other university.

Alexandra Yashina
Abstract

Research into the crystallisation of calcium carbonate traverses many disciplines, including chemistry, physics, biology and materials science. Control over the polymorph, hierarchical assembly, orientation, size and shape has been the focus of significant interest in developing new applications. Current synthetic techniques offer limited control over the crystallisation process and often require high temperatures and/or pressures and the use of organic additives and functionalised templates.

Microfluidic systems offer superior control over reaction conditions when compared to traditional macroscale methods and hence provide an alternative approach for the synthesis of particles. Not surprisingly, the literature includes hundreds of papers reporting the microfluidic synthesis of nanomaterials. The control provided by microfluidics allows the arrest of a reaction at any particular time, which is hugely advantageous when studying the nucleation and crystallisation of such particles. Moreover, since most biochemical reactions occur in aqueous media, droplet-based microfluidic devices are excellent tools for the miniaturisation of reaction volumes and the simulation of natural or synthetic processes.

In the first part of this thesis, custom built droplet-based microfluidic systems are applied to the study of the precipitation of calcium carbonate under highly controlled conditions. Reactions performed in pL-volume droplets dispersed within a continuous carrier fluid afford reproducible control over crystal polymorph, such that pure calcite, pure vaterite or a mixture of calcite and vaterite can be preferentially precipitated by varying the concentration of reagents. This contrasts with poor reaction control on the macroscale.

In the second part of this thesis the early stages of the nucleation of amorphous calcium carbonate are studied in similar droplet-based formats. The precipitation of calcium carbonate is arrested at various time points, with a maximum residence time of 2 minutes, and amorphous calcium carbonate is analysed over different growth periods. Additionally, the effect of poly(styrene sulphonate) on the precipitation and growth of amorphous calcium carbonate is investigated. Results show that in the presence of polymer, calcium carbonate growth occurs by mesoscale assembly rather than by classical nucleation.
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List of Publications

1) Microfluidic Reactors for Nanomaterial Synthesis

S. Krishnadasan, A. Yashina, A.J. deMello and J.C. deMello *Advances in Chemical Engineering* 2010, 38, 195

2) Calcium carbonate polymorph control using droplet-based microfluidics

A. Yashina, F. Meldrum, A. deMello *Biomicrofluidics*, 2012, 6, (2), 022001
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<tbody>
<tr>
<td>A</td>
<td>Interfacial Area</td>
</tr>
<tr>
<td>ACC</td>
<td>Amorphous Calcium Carbonate</td>
</tr>
<tr>
<td>AFM</td>
<td>Atomic Force Microscopy</td>
</tr>
<tr>
<td>AP</td>
<td>Activity Product</td>
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<tr>
<td>Ca</td>
<td>Capillary Number</td>
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<tr>
<td>CNT</td>
<td>Classical Nucleation Theory</td>
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<tr>
<td>D</td>
<td>Molecular Diffusion Coefficient</td>
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<tr>
<td>EWOD</td>
<td>Electrowetting-on-dielectric Device</td>
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<tr>
<td>Fo</td>
<td>Fourier number</td>
</tr>
<tr>
<td>G*</td>
<td>Maximum Free Energy</td>
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<tr>
<td>HR</td>
<td>High Resolution</td>
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<tr>
<td>K</td>
<td>Indeterminate Kinetic Constant</td>
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<tr>
<td>Ksp</td>
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<tr>
<td>Δμ</td>
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<td>μ</td>
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<td>μI</td>
<td>Chemical Potential in the Bulk Phase</td>
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<td>μ</td>
<td>Viscosity of the Fluid</td>
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<tr>
<td>η</td>
<td>Fluid Viscosity</td>
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<tr>
<td>n</td>
<td>Number of Molecules in the Embryo</td>
</tr>
<tr>
<td>ρ</td>
<td>Density of the Fluid</td>
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<tr>
<td>PDMS</td>
<td>poly(dimethylsiloxane)</td>
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<tr>
<td>PSS</td>
<td>poly (4-sodium styrene sulfonate)</td>
</tr>
<tr>
<td>PTFE</td>
<td>Poly(tetrafluoroethylene)</td>
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<tr>
<td>PFPE</td>
<td>Perfluorinated polyether</td>
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<tr>
<td>Symbol</td>
<td>Description</td>
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</tr>
<tr>
<td>PEG</td>
<td>Polyethylglycol</td>
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<td>$Q_w$</td>
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<td>$Q_o$</td>
<td>Continuous Phase Flow Rate</td>
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<tr>
<td>$q$</td>
<td>Energy of Diffusion across the Interface</td>
</tr>
<tr>
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<td>Reynolds Number</td>
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<td>SEM</td>
<td>Scanning Electron Microscopy</td>
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<td>SAM</td>
<td>Self Assembled Monolayer</td>
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<td>$\delta$</td>
<td>Hydrodynamic Diameter of the Channel</td>
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<tr>
<td>$t_{diff}$</td>
<td>Average Diffusion Time</td>
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<td>Velocity of the Flowing Fluid</td>
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<td>Water Fraction</td>
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<td>Characteristic Dimension</td>
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Chapter 1

Background and Theory

This chapter provides an overview of the chemistry and synthesis of calcium carbonate and the advantages of using droplet-based microfluidic devices for its production. The use of droplet-based microfluidic reactors in synthetic chemistry is also introduced. The advantages and challenges of particles synthesis using both continuous and segmented flow microfluidics is described with reference to previous work.
1.1 Introduction

The field of crystallisation crosses many disciplines, including chemistry, biology, physics and material science. Control over the size, orientation, organisation, morphology and hierarchical assembly of particles has been of importance in the design of new materials across a wide range of fields, including pharmaceutical science,[1,2] medicine,[3] electronics,[4-6] food technology[7] and ceramics[8].

Present synthetic techniques offer limited control over the crystallisation process and frequently require high pressures and temperatures or the use of expensive screening methods to control the properties of the resulting particles. The control of morphology and surface features of such materials remains an immense technical challenge. Recent strategies for the synthesis of inorganic materials with controlled morphologies include the use of functionalised templates,[9,10] self-assembling organic superstructures[11] and organic additives.[12] These biomimetic approaches are inspired by nature, and aim to develop new synthetic methodologies through the study of biomineralisation mechanisms.

Biomineralization is a natural process by which living organisms secrete inorganic minerals in the form of teeth, shells, skeletons etc. Biominerals are highly structured and optimized materials with function-specific properties, and have attracted a lot of attention as natural archetypes for future materials.[13] Biomineralisation offers outstanding control over the crystallinity, morphology, composition and material properties at physiological temperature, pressure and pH.[14] For example Figure 1.1 presents SEM micrographs of nacre from molluscs. Nacre is a mineralised tissue deposited by many mollusc species to build the inner layers of their shells.[15] It is very strong, and composed of hexagonal platelets of an aragonite polymorph of calcium carbonate arranged in continuous parallel lamina.

The biomineralisation phenomenon is extremely widespread in nature and occurs in a variety of organisms such as exoskeletons in protozoa, skeletal plates of echinoderms, mammalian bone and teeth, eggshells and stratoliths.[16] High spatial control in biomineral formation is achieved through the use of confined reaction environments, where the shape of inorganic crystals is usually related to the intrinsic unit cell structure.[17] This is difficult to replicate
under laboratory conditions. Consequently, a better understanding of nucleation is fundamental to controlling crystallisation processes, as it defines features of the final product such as size, crystallinity and morphology.

Figure 1.1  (A, B) SEM micrographs of nacre from the bivalve *A. rigada*. (A) Fracture section of *Atrina* nacre consisting of thin (30 nm) layers of matrix alternating with 500 nm layers of aragonite polymorph of calcium carbonate. Arrows indicate two layers. (B) Fracture section of *Nautilus* nacre consisting of 60-70 nm layers (see arrows). Image taken from reference [15]

In recent years, microfluidic devices have emerged as an attractive technology for particle synthesis, as they offer superior levels of reaction control than achievable in conventional macroscale systems.[18] Over the past two decades microfluidic systems have been shown to possess many features ideally suited to the processing of chemical reactions. These include fast processing times, facile automation, high analytical throughput and reproducibility.[18,19] Whilst microfluidic technologies have been used before to perform protein crystallisation [20-25] and nanoparticle synthesis [26-32] their application to the crystallisation of other materials such as inorganic species, has been surprisingly limited. In this thesis, droplet-based microfluidics is applied to the study of calcium carbonate crystallisation.
1.2 Crystal Formation

The basic process of crystallisation comprises three distinct stages, supersaturation, nucleation and growth. An overview of each process is now presented.

1.2.1 Supersaturation and the Metastable Zone

Supersaturation is the requisite driving force for crystallisation.[33] It occurs when the amount of dissolved material is greater than the solubility limit. Supersaturation results from a physical change to the analytical solution, most often in temperature, pressure or chemical composition and was first described by Lowitz in 1795.[33] Subsequently, Gibbs stated that in supersaturated solutions, the free energy of the initial solution is greater than that of the crystalline phase plus that of the final solution phase.[34,35] Hence, supersaturation is often expressed in terms of a change in the chemical potential, $\Delta \mu$, as this is a measure of the change in free energy upon phase transformation:

$$\Delta \mu = -k_B T \ln \frac{AP}{K_{sp}}$$

(1.1)

Here $k_B$ is the Boltzmann constant, $T$ is the absolute temperature, $AP$ is the activity product of the reactant and $K_{sp}$ is the equilibrium activity product. $AP/K_{sp}$ is equal to the relative supersaturation, $\sigma$, which is directly proportional to the difference in Gibbs free energy between the bulk phase and the nucleating phase. Since

$$\sigma = \frac{AP}{K_{sp}}$$

(1.2)

$\Delta \mu$ is expressed as,

$$\Delta \mu = -k_B T \ln \sigma$$

(1.3)

First stated by De Coppet, there is a finite region of supersaturation within which crystallisation occurs spontaneously via nucleation and growth.[36] This metastable zone, is called the Ostwald-Miers zone and is specific for a given solution.[36,37] Ostwald observed a
sharp boundary between metastable and unstable solutions, which is approximately parallel to the solubility curve. This boundary is called the *supersolubility curve* and divides the solubility diagram into three zones: the unstable zone, metastable zone and stable zone (Figure 1.2). The width of the metastable zone is important in controlling nucleation and the resultant crystal, since size distribution, polymorphism and morphology are all dependent on the degree of metastability.[38] In practice, the metastable zone is much narrower than predicted, as the presence of impurities, vessel size and type, solution agitation rate and cooling rate have an effect on the metastable zone width.[38] The width of the metastable zone can be determined experimentally by either isothermal or polythermal methods.[36] In the isothermal method, supersaturation is achieved by evaporating at a constant rate and temperature. The polythermal method, however, is based on using a constant rate of cooling to create supersaturation without changing the concentration. Understanding the metastable zone width is important in industrial crystallizers as they are operated under supersaturation conditions in the metastable zone to obtain the required size, particle size distribution and shape of the crystals.[38]

![Figure 1.2](image.png) Figure 1.2 The solubility state as a function of solute concentration (c) and temperature (T). The solution is stable below the solubility curve and unstable above the supersolubility curve. The metastable phase exists between the two curves. Image taken from reference [36].
Theoretical studies aimed at predicting the metastable zone width from nucleation kinetics have been successful in specific systems. For example, Kaschiev et al. [39] related the width of the metastable zone to the nucleation induction time. Nyvlt proposed a method that relates the order of nucleation to the experimentally determined $\Delta T_{\text{max}}$ and cooling rate with an assumption that when the nuclei are first detected, the generation rate of supersaturation is equal to the rate of nucleation.[40] However, to date, no universal model for predicting the metastable zone, under all conditions has been found. This has resulted in low levels of control in synthetic crystallisation processes.

1.2.2 Nucleation

During nucleation, a molecular aggregate of a new phase begins to form within the mother (metastable) phase.[41] In order for nucleation to occur, the free energy barrier must be exceeded, allowing the formation of nuclei of a critical size.[42,43] The lower free energy of the new phase drives the formation of the nuclei and is proportional to the volume of the nuclei. The process is hindered, however, by the energy required to form an interface between the nucleus and the metastable phase, which is proportional to the surface area of the nuclei. Thus, only nuclei larger than a critical size will grow spontaneously, whereas smaller nuclei will dissolve back into the metastable phase. Homogeneous nucleation occurs within the bulk solution in the absence of nucleation sites (such as impurities and the walls of the container). Heterogeneous nucleation occurs in the presence of impurities and solid surfaces, which provide preferential nucleation sites by lowering the free energy nucleation barrier. Classical nucleation theory (CNT) describes both processes and was originally presented by Volmer and Webber in 1926.[44]

1.2.2.1 Homogeneous Nucleation

Homogeneous nucleation is a highly localised process and begins with the association of atoms due to statistical density fluctuations.[45] An interface is formed between the small “embryos” of the new phase and the surrounding metastable bulk phase. Frequent collisions
occur on an atomic scale between the embryo and the bulk phase, with the resultant energy dissipating via translational, vibrational and rotational modes of the embryo.[46] Occasionally, the molecule either leaves the embryo (fission) or attaches to the embryo (aggregation) due to an inelastic collision. These events occur in competition with each other, where fission is more frequent than aggregation. Thus, it is only occasionally that the embryo reaches the size of the critical nucleus. The size of the critical nucleus primarily depends on temperature, molecular volume, relative supersaturation and interfacial energy.[47] Classical homogeneous nucleation theory defines \( J \) as the number of nuclei forming per unit time per unit volume. Volmer and Weber,[44] and later Becker[48] proposed the following expression for \( J \),

\[
J = K \exp\left(-\frac{G^* + q}{k_B T}\right)
\]  

Here, \( G^* \) is the maximum free energy required for the formation of critical nuclei, \( q \) is the energy of diffusion across the interface, \( k_B \) is the Boltzmann constant, \( T \) is the absolute temperature and \( K \) is a kinetic constant.

### 1.2.2.2 Heterogeneous Nucleation

As noted the presence of nucleation sites decreases the energy barrier for embryo formation, leading to a much faster rate of nucleation than predicted.[49] This is because the interfacial energy between a nucleus and a substrate species is lower than when the nucleus in contact with the solution. This leads to the formation of bonds with the substrate that are stronger than the bonds of solvation.[35] The bond strength depends on the atomic structure and chemistry of the substrate surface. When the atomic structure of the surface of the substrate matches that of a particular plane of the nucleating phase, the lattice strain in minimised and strong bonding to the nucleus is promoted on that crystal plane.[35] When a liquid droplet phase forms from supercooled vapour, the embryo-substrate interfacial energy is almost always lower than the embryo-bulk interfacial energy. The embryo wets the substrate, with more wetting corresponding to a lower nucleation barrier. The minimum work of formation for a nucleus growing on a substrate is given by:
\[ W_{\text{min}} = \Sigma_{bc} A_{bc} + (\Sigma_{bs} - \Sigma_{cs}) F_{bs} + (P - P^I) V^I + [\mu^I(T, P^I) - \mu(T, P)] n \]  

(1.5)

Here, \( A \) is the interfacial area and \( \Sigma \) is the surface tension of the interfaces, where subscript \( b \) denotes the bulk phase, \( c \), the crystalline phase and \( s \), the substrate. \( n \) denotes the number of molecules in the embryo and \( \mu \) and \( \mu^I \) are the respective chemical potentials in the embryo and bulk phases. \( P \) and \( P^I \) are the pressures of the bulk and the embryo phase respectively.

1.2.3 Crystal Growth

Growth from a supersaturated solution occurs when the flux of molecules attaching to the crystal surface is greater than the flux of molecules detaching from the surface. The ability of a molecule to detach from a crystal is determined by the strength of its bonds to that crystal.[35] Both kinetic and thermodynamic effects influence the growth of the crystal and are discussed below.

1.2.3.1 Thermodynamic Crystal Growth

Based on thermodynamics, the surface energy of the crystal faces and the external growth environment define the morphology of a crystal.[17] The fast growing faces with high surface energies will disappear in the final morphology and \textit{vice versa}. Upon intentionally lowering the surface energy of specific faces, anisotropic growth of the crystal can be achieved.[17] Ions or organic additives can adsorb to specific crystal faces, lowering their surface energy, and hence inhibiting growth. Although a purely thermodynamic approach cannot predict the crystal morphology, it is usually used to explain the additive-mediated morphology changes because growth of specific crystal faces can be assessed.[17]
1.2.3.2 Kinetic Crystal Growth

Kinetic crystallisation is based predominantly on the modification of the activation energy barriers of nucleation, growth and phase transformation.[50] Unlike thermodynamic crystal growth, which proceeds in a single step, kinetic growth is a sequential process involving modifications of the amorphous precursors and crystalline intermediates.[50-52] These modifications occur by dissolution-recrystallization processes and are closely associated with the characteristics of the preformed particles.[17] Consequently, kinetically driven crystallization frequently involves the initial formation of an amorphous phase. This phase is hydrated and is susceptible to rapid phase transformations. Control of kinetic crystallisation can be achieved by altering the interactions of the nuclei and developing crystals with solid surfaces or soluble molecules. This in turn changes the structure and composition of the nuclei, particle size, texture, ability to aggregate and stability of the intermediate phases.[17] Figure 1.3 shows the energy profile associated with both kinetic and thermodynamic growth routes.

Figure 1.3  Crystallisation pathways under thermodynamic (A) and kinetic (B) growth. The choice of route depends on the free energy of activation ($\Delta G$) associated with nucleation (n), growth (g) and transformation (t). Image taken from reference [50].
1.3 Calcium Carbonate

Half of all known biominerals contain calcium cations \([\text{Ca}^{2+}]\), such as calcium phosphates, calcium oxalate and calcium carbonate.[53] Calcium carbonate is one of the most abundant biominerals and possesses mechanical properties superior to most other composite ceramics in terms of stiffness, strength and toughness.[54] CaCO_3 has recently received a great deal of attention due to its use in the adhesives, rubber, paper, paints and plastics industries.[54] Biominerals of calcium carbonate are classified into three categories: amorphous, polycrystalline and single crystal.[55] Amorphous minerals do not possess a regular internal atomic structure (Figure 1.4 C) and are metastable with respect to the polycrystalline and crystalline material.[13] However, when formed under biological control, the amorphous materials are reportedly stable for the lifetime of the organism.[56] Since amorphous materials do not have a defined form, they can be readily moulded into a desired shape by the organism.[57,58] Polycrystalline biominerals exhibit a wide range of morphologies and are assembled from small crystalline units of varying size and orientation (Figure 1.4 B).[59] Single crystal biominerals exhibit a continuous and unbroken crystal lattice (Figure 1.4 A) and the absence of defects enables them to have unique properties such as high mechanical strength.[59]

![Figure 1.4](image_url)  
Schematic showing the arrangement of particles in (A) crystalline, (B) polycrystalline and (C) amorphous materials.
CaCO₃ exists in three anhydrous polymorphs; calcite, aragonite and vaterite, as well as a highly unstable amorphous phase; amorphous calcium carbonate (ACC). A schematic of polymorph transformations starting from unstable ACC are shown in Figure 1.5.

![Schematic showing the transformations of CaCO₃ polymorphs. The amorphous phase readily transforms into metastable vaterite, which in turn transforms either into thermodynamically stable calcite or aragonite. Aragonite is metastable with respect to calcite. ACC can be directly transformed into calcite at low solution supersaturations.](image)

1.3.1 Amorphous Calcium Carbonate

The existence of amorphous calcium carbonate was first reported over 100 years ago when it was observed that certain calcium carbonate deposits were isotropic when viewed between cross polarised lenses.[16,60] It was later shown that these deposits did not diffract X-rays, a basic characteristic of a crystalline material.[61] ACC is highly soluble and has been described as having the composition CaCO₃.1.5H₂O.[62] It is known to be a precursor phase during the process of biomineralisation,[54] however it readily transforms into a more stable polymorph of CaCO₃ unless kinetically stabilised. During biomineralisation, this stabilisation is known to be achieved by ions, such as Mg²⁺ and PO₄³⁻,[16] or by enclosing ACC in an non-permeable sheath of organic macromolecules.[63] Synthetic ACC can be stabilised using various additives, including amino acids and polyphosphates. It is therefore of significant interest to be able to generate ACC synthetically and mould it into required polymorph of specific size by use of external molecules or a template.
1.3.2 Calcite

Calcite is the most thermodynamically stable polymorph of CaCO$_3$ and hence the most abundant carbonate material. It exhibits a hexagonal (rhombohedral) structure and is either precipitated directly from the solution (at low supersaturations) or via the dissolution of a precursor phase (ACC, vaterite or aragonite). The structure of calcite is often compared to that of NaCl, where the Na$^+$ and Cl$^-$ ions are replaced by Ca and CO$_3$ groups respectively.
1.3.3 Aragonite

Aragonite is the second most stable and common form of CaCO$_3$. It is usually metastable with respect to calcite and only forms within a narrow range of conditions. Aragonite is denser and harder than calcite and precipitates to form needle-like crystals. It is commonly found in nature[13] and can be synthetically precipitated at high temperatures.[68]

![SEM micrographs of aragonite crystals precipitated at (A) 95 °C and (B) 80 °C. Images taken from references [69] and [67] respectively.](image)

1.3.4 Vaterite

Vaterite is metastable with respect to calcite and aragonite and is extremely rare in nature.[54] However, it is a common synthetic product of solution precipitations and readily transforms into calcite or aragonite at low and high temperatures respectively.[70-72] Precipitation of vaterite is favoured by increasing the rate of precipitation, lowering the temperature and by adding certain additives which stabilise the metastable phase.[54] When not stabilised by additives, vaterite forms spherical particles and rapidly transforms into calcite.[73]
1.4 Microfluidic Systems as Synthetic Tools

Microfluidic systems were first introduced into the scientific world about three decades ago.[75] Since then, the technology has developed rapidly and found exciting applications in numerous fields,[18,76] such as chemical synthesis,[77] biochemical analysis[77] and drug discovery.[78-80] The advantages presented by miniaturisation include ultra-small sample volumes, low instrument cost, portability, reduced analysis times and improved analytical performance.[18] One of the major features of microfluidic systems is the importance of viscous forces and the lack of turbulence. In macroscopic systems, fluids usually mix convectively, with inertial forces controlling mass-transfer timescales. In microfluidic systems, fluids normally flow in parallel, without turbulence, and mixing occurring via molecular diffusion across fluidic interfaces.

Before a reaction between two reagents can occur, an intimate contact between the molecules must be established through mixing. On a microfluidic scale, fluids do not mix convectively and mixing occurs via diffusion across fluidic interfaces.[18] In simple terms this diffusional mixing timescale can be characterised using Einstein-Smoluchowski equation;

$$t_{\text{diff}} = \frac{x^2}{2D}$$  \hspace{1cm} (1.6)
Here, \( t_{\text{diff}} \) (s) is the average diffusion time, \( x \) (m) is the characteristic dimension and \( D \) (m\(^2\)s\(^{-1}\)) is the molecular diffusion coefficient. Since mixing can only be accomplished by diffusion, rather than through convection (dominant in turbulent systems) the diffusional timescale has a direct effect on the efficiency of the reaction and product distribution. Hence, it is normally important that the reaction proceeds within the chemical regime where diffusion is fast compared to the reaction rate. Formation of secondary products is more likely to occur if the reaction proceeds in the diffusional regime where the reaction is fast and diffusion is the rate-limiting step.[18]

Diffusive mixing efficiencies can be assessed using the Fourier number, i.e.

\[
F_o = \frac{D t_{\text{diff}}}{L_{\text{mixing}}^2}
\]

(1.7)

Here, \( D \) is the diffusion coefficient, \( t_{\text{diff}} \) is the average diffusion time over the characteristic mixing length \( L_{\text{mixing}} \). Generally, a \( F_o \) greater than 1 indicates complete mixing, values between 1 and 0.1 indicate adequate mixing and values below 0.1 indicate poor mixing.

In microfluidic reactors, the flow regime is most easily characterised through assessment of the Reynolds number.[18,76] The Reynolds number is a dimensionless number assessing the ratio of inertial forces to viscous forces:

\[
Re = \frac{\delta \rho v}{\eta}
\]

(1.8)

Here, \( Re \) is the Reynolds number, \( \delta \) (m) is the hydrodynamic diameter of the channel, \( \rho \) (kgm\(^{-3}\)) is the density of the fluid, \( v \) (ms\(^{-1}\)) is the velocity of the flowing fluid and \( \eta \) (kgm\(^{-1}\)s\(^{-1}\)) is the fluid viscosity. As a rule of thumb turbulent flow occurs when Reynolds numbers are greater than 2000. In microfluidic systems, Reynolds numbers are typically much less than 2000, indicating a laminar flow regime.

1.4.1 Continuous and Segmented Flow Regimes

In broad terms, microfluidic systems operate within a single-phase or multi-phase flow regime. A significant problem encountered in single-phase microfluidic systems is the
existence of a parabolic velocity distribution, with fluid velocity being near zero at the channel walls and maximum at centre (Figure 1.10 A). Parabolic flows are caused by channel walls applying shear forces on the fluid, and cannot be avoided. If a reaction mixture is introduced into a microfluidic channel and motivated a given distance using a hydrodynamic flow, the resulting reaction mixture will consist of elements that have spent varying times on-chip. This leads to a residence time distribution and consequently variations in product distribution of the reaction.[18] Conversely, in segmented flow regimes, discrete liquid droplets formed in microchannels serve as confined reaction environments and are encapsulated by an immiscible carrier fluid (Figure 1.10 B).[18] Droplets can be generated at kHz frequencies and are analogous to miniaturised reaction chambers, separated from each other and the device walls by a continuous phase. Droplets move along the channel at a constant velocity, thus minimising the velocity distributions present in continuous-flow systems.

\[
Ca = \frac{U \mu}{\gamma}
\]  

(1.9)

1.4.2 Droplet Formation in Microfluidic Systems

Droplet formation in planar, chip-based microfluidic systems is normally achieved using one of two methods involving fluidic T-junctions (Figure 1.11 A) or flow-focusing geometries (Figure 1.11 B).[18] Droplet formation can be assessed using the Capillary number (\(Ca\)),
Here, $Ca$ is the capillary number, $U\, (ms^{-1})$ is the flow velocity, $\mu\, (kgm^{-1}s^{-1})$ is the viscosity of the fluid and $\gamma\, (Nm^{-1})$ is the surface tension between the droplet fluid and the carrier fluid. Low values of $Ca\, (< ~ 0.1)$ ensure stable droplet formation.[82] High values of $Ca\, (> ~1)$ induce break-up of droplets, so that the droplet diameter is much smaller than the channel width.

![Figure 1.11](image)

(A) Droplet generation at a T-junction. (B) Droplet generation by flow-focusing. For both devices, the channel width is 100 $\mu$m and height is 50 $\mu$m.

### 1.4.2.1 Flow-Focusing Method

In a flow-focusing geometry, the continuous phase is injected through two outside channels and the dispersed phase is injected through a central channel (Figure 1.11 B). All streams (continuous and dispersed phases) are then forced to flow through a small orifice. The continuous phase exerts pressure and viscous stress which force the dispersed phase to form a narrow thread, which then breaks inside or downstream of the orifice, creating droplets.[83]

Droplet size can be controlled by varying the relative flow rates of the two phases and is quantified as a function of the ratio of the internal dispersed phase flow rate to the external continuous phase flow rate, $Q_i/Q_o$.[83] To generate large droplets, one can either decrease the flow rate of the continuous phase and/or increase the flow rate of the dispersed phase. Anna et al.[83] studied droplet formation in a flow-focusing geometry and published a phase diagram showing the variation of droplet size as a function of flow rates and flow rates ratios of the two phases (Figure 1.12).
Phase diagram showing droplet formation in flow-focusing geometry by varying the flow rate of oil phase, $Q_o$ and $Q/Q_o$. Droplets of varying sizes are produced. High oil flow rates result in formation of droplets much smaller than the orifice width (k) and (q). Image taken from reference [83]

1.4.2.2 T-Junction Method

In this approach the continuous and dispersed phases are injected from two orthogonal branches of a T-junction (Figure 1.11 A).
In this situation, the pressure and shear forces generated by the continuous phase force the head of the dispersed phase to elongate into the main channel until it is thin enough to break off, creating a stream of droplets.[81] The size of the formed droplets can be varied by changing the channel or inlet widths, fluid flow rates, or the viscosities of the two phases. The water fraction, $W_f$, is used to characterise the relationship between the fluid flow rates and droplet size, where

$$W_f = \frac{V_{aq}}{V_{aq} + V_o}$$  \hspace{1cm} (1.10)

Here, $W_f$ is the water fraction, $V_{aq}$ ($m^3s^{-1}$) is the aqueous phase flow rate and $V_o$ ($m^3s^{-1}$) is the continuous phase flow rate.

### 1.4.3 Carrier Fluid in Microfluidic Systems

The most important factor in allowing the creation of stable and monodisperse droplets is the choice of the oil carrier fluid. In order for droplets to form successfully, the carrier fluid must be immiscible with the aqueous phase and must preferentially wet the microchannel surface. Fluorinated oils, such as FC40 and FC70 (3M), are widely employed as carrier fluids in droplet-based microfluidic reactions, as they are hydrophobic and immiscible with aqueous solutions as well as organic solvents. They are also compatible with PDMS (polydimethylsiloxane), a common material used to make microfluidic devices, and compatible with biological molecules.[84] Nevertheless, other oils such as dodecane, hexadecane, and mineral oil have also been successfully used for droplet generation.[85,86]

For droplets to be fully functional microenvironments there must be no cross-contamination between them. As droplets are prone to coalescence, surfactants (added to the continuous phase) are crucial in ensuring that droplets remain stable throughout the experiment. They also ensure that sample does not adsorb at the interface of the droplet, and prevent sample evaporation during long term droplet storage. Surfactants such as Span-80 (Sigma-Aldrich), Krytox (DuPont), Raindance (Raindance Technologies) and 1H,1H,2H,2H-perfluorooctanol have all been successfully employed in droplet-based microfluidic experiments.[87,88] It is
therefore important to choose the correct oil/surfactant combination to meet the requirements for a particular application in droplet-based microfluidics.

### 1.4.4 Droplet Manipulations

In order for droplets to function as microreactors, droplet manipulation post generation is desirable. Several techniques have been developed in recent years to allow droplet mixing, fusion, sorting, dilution and splitting.[89-91] Such operations are either performed using passive or active mechanisms. Passive control is achieved using variations in surface chemistry or device geometry and active control through use of external stimuli such as electric fields.[92] Mixing is a prerequisite for a reaction to occur between two reagents and, in continuous flow microfluidic systems, is achieved by the diffusion of reagents across fluidic interfaces. In a droplet moving along a straight channel, mixing occurs by recirculation of fluid. In each droplet, two vortices are formed and solutions are mixed in the right and left halves of the droplet (Figure 1.13).[93] If the reagents are located in the front and back halves of the droplets, then the flows recirculate within the left and right halves resulting in efficient mixing (Figure 1.13 B). However if reagents are located in the left and right halves of the droplet, the recirculating flow does not result in efficient mixing (Figure 1.13 A).

![Diagram](image.png)

Figure 1.13 (A) Two vortices formed within droplet moving through a straight channel. Solution is only mixed in the right and left halves of the droplet. (B) Reagents located in the front and back halves of the channel result in efficient mixing as flow recirculates within the droplet. Image taken from reference [82].
If unsteady fluid flows can be generated inside the channels, mixing can be significantly enhanced by chaotic advection.[82] To enhance internal mixing within droplets, the channel geometry can be modified to create chaotic advection that allows folding, stretching and reorientation of strata within droplets (Figure 1.14).[81]

![Image of winding channels with aqueous droplets. The channel width and height are 100 and 50 µm respectively. (B) Model demonstrating the mixing of two components by chaotic advection within droplets. Image taken from reference [81].](image)

Droplet coalescence can also be achieved using active or passive approaches. For instance, active droplet coalescence has been achieved through application of an electric field parallel to the droplet channel.[92] Application of a low voltage (between 1 and 3 V) applied to electrodes patterned across a microfluidic channel induced instability across the interface between the droplets, resulting in droplet merging (Figure 1.15 A).[92] Passive and controlled droplet merging can also be achieved by incorporating a specially designed “merging chamber”, consisting of an array of pillars into a channel (Figure 1.15 B).[89] Here the array of pillars causes an initial droplet to slow down, thus allowing a following droplet to come close enough to merge by draining the oil phase between them. Using this approach,
the number of droplets to be merged can be controlled by varying droplet size, array length and pillar spacings.

Figure 1.15 (A) Targeted electrocoalescence of droplets. Electrodes patterned across channel walls cause a dynamic instability across the interface of the neighbouring droplets, causing them to merge. (B) Pillar structure promoting the coalescence of two aqueous droplets. Images taken from references [92] and [89] respectively.

Monitoring reactions in droplets over an extended time period can be achieved by storing them in a way that prevents coalescence. For example, Huebner et al. incorporated a static trap array within a microfluidic device that can be used to store hundreds of picoliter droplets simultaneously (Figure 1.16 A).[91] In addition, Holtze et al. synthesized a novel class of fluorosurfactants for stabilising oil-in-water emulsions (Figure 1.16 B).[88] The authors coupled oligomeric perfluorinated polyethers (PFPE), to provide good stabilisation of water-in-fluorocarbon emulsions, with polyethyleneglycol (PEG), to prevent adsorption of biological material. Such surfactants not only maintain droplet stability, but also are compatible with biological molecules and cells.

Figure 1.16 (A) Droplet storage achieved by array of traps. (B) Droplet storage achieved by introducing a fluorosurfactant into the oil phase. Images taken from references [91] and [88] respectively.
A recent advance in droplet manipulation involves the realisation of a droplet dilutor, which enables rapid and controllable alteration of the sample concentrations within a train of droplets.[90] In this work, a “mother” droplet containing a reagent is trapped inside a microfluidic chamber. Successive input buffer droplets merge with this mother droplet and generate a series of droplets with concentrations decreasing exponentially according to the \((1 - V_d/V_M)^n\) where \(n\) is the droplet number, \(V_d\) is the volume of the output droplet and \(V_M\) is the volume of the “mother” droplet (Figure 1.17).

![Figure 1.17](image.png)

**Figure 1.17** Process of sequential dilution where a “mother” droplet is localised inside a trapping chamber and successive buffer droplets are merged with it to create a series of droplets with concentrations decreasing exponentially. Image taken from reference [90].

### 1.4.5 Applications of Microfluidic Reactors in Nanoparticle Synthesis

Nanoparticles exhibit physical and chemical properties that are largely dependent on size, shape and structure. Accordingly precise control of the synthetic process is necessary to generate particles of the desired characteristics.[94] Briefly, rapid initial mixing of the
reagents is required to induce supersaturation and the formation of “seed” nuclei. Nuclei smaller than a critical size dissolve whilst those larger, grow at the expense of the precursor particles. In most situations, nucleation and growth occur concurrently throughout particle formation, resulting in a broad distribution in particle size. In addition, the size and shape of produced nanoparticles will depend on precursor concentrations, reaction temperature, residence time and additives. Once a growing particle has reached a desired size, the reaction is quenched before the particles have a chance to aggregate. Particle properties are therefore influenced by control over mixing and temperature profiles during synthesis, as well as variations in the initial concentrations of reagents. Mixing in microfluidic systems is typically both faster and more controlled than in bulk systems due to short diffusion distances. Moreover in macroscale synthetic systems significant variations in reagent concentrations occur in both time and space due to poor mixing. Both single-phase and droplet-based microfluidic systems have been used for nanoparticle synthesis and have proved to have numerous advantages over bulk synthetic methods. These include superior control of particle size, shape, morphology and size distribution. The following examples of the microfluidic synthesis of inorganic materials highlight these advantages.

1.4.5.1 Microfluidic Synthesis of Compound Semiconductor Nanoparticles

Compound semiconductor nanoparticles (or quantum dots) were the first nanoparticles synthesised in microfluidic systems. The optical and electronic properties of quantum dots are related to their size and shape. Their tunable band gap, which normally lies within the visible range of the electromagnetic spectrum, is determined by the physical size and shape of the nanocrystallites. The continuous-flow microfluidic synthesis of CdS nanoparticles was first reported by Edel et al. 10 years ago. Aqueous solutions of Cd(NO$_3$)$_2$.4H$_2$O and Na$_2$S were mixed in the presence of a sodium polyphosphate stabiliser. The product obtained had a narrower size distribution than that achieved using the corresponding bulk method. Subsequently, a high-temperature droplet-based synthesis of high quality CdSe nanocrystals was demonstrated by Chan et al. The authors used octadecene as the discrete phase and a high boiling-point perfluorinated polyether as the carrier phase. Synthesised nanoparticles
had diameters of 3.4 nm and were formed when the reagents were heated to 290°C. Many more articles have since been published in this area, and in virtually all cases clear advantages were found over the corresponding bulk synthesis, especially in the ability to tune the properties of the final product.[98-101] More specifically, when comparing droplet-based methods with continuous-flow methods, it has been shown that the size distribution of the produced particles is narrower due to the absence of residence time distributions inherent in pressure-driven flow.[102] Moreover, in continuous flows there is a tendency for the formed particles to nucleate and deposit on channel walls, leading to blockages of the channels or unstable reaction conditions.[27]

1.4.5.2 Microfluidic Synthesis of Metal Nanoparticles

The catalytic properties of metal nanoparticles are size and shape dependent and have received considerable attention because of their broad range of applications.[103] A variety of metal nanoparticles have been synthesised in microfluidic reactors. For example, single-phase microfluidic flows have been used to synthesise gold nanoparticles from gold salts (HAuCl₄) and ascorbic acid over a range of pH values, reactant flow rates and concentration ratios.[29] The size distribution of the prepared particles was on average two times narrower than that achieved using the corresponding bulk method. Recently, different shapes of gold nanoparticles have been synthesised in a droplet-based microfluidic device from small, spherical gold seeds.[104] Droplets were generated at a T-junction and silicone oil used as the carrier phase (Figure 1.18 A, B). Different shapes were achieved by tuning the concentration of the reagents, which were composed of gold seeds (S) and solutions of Au³⁺ (R1) and reducing agents (R2). The Au³⁺ solution also contained small amounts of Ag⁺ to enhance the growth rate of different crystal facets and encourage anisotropic growth. The products ranged from spherical and spheroid nanoparticles to rod-like and extended sharp-edged shapes depending on the concentration of Ag⁺ used (Figure 1.18 C, D, E).
In another example, copper nanofluids synthesised in a single-phase microfluidic device had an average size of 3.4 nm with a coefficient of variation of 22%. [28] The same synthesis carried out in the bulk generated nanoparticles with the sizes ranging from 2.7 to 4.0 nm, with a coefficient of variation larger than 30%. Moreover, the reaction time in bulk synthesis was 10 minutes whereas that in microfluidic synthesis was 28 seconds. Similarly, palladium nanoparticles were synthesised in a single-phase microfluidic flow and had a mean diameter of 3.0 nm with the relative standard deviation of 10%. [105] Bulk synthesis, on the other hand, obtained nanoparticles of 3.2 nm with the relative standard deviation of 35%.

1.4.5.3 Microfluidic Synthesis of Metal Oxide Nanoparticles

Metal oxide nanoparticles have a broad range of applications due to their optical, magnetic and electrical properties. [106] For example, iron oxides are widely used as contrast enhancement agents in magnetic resonance imaging due to their magnetic properties. [107]
and for drug delivery. Controlling the synthesis conditions when making such particles is crucial since they determine their magnetic properties. The first droplet-based microfluidic synthesis of iron oxide nanoparticles was reported in 2008 by Frenz et al. The fusion of droplets containing the required reagents was achieved by electrocoalescence using two embedded electrodes (Figure 1.19 A). The average particle diameter synthesised in droplets was 4 ± 1 nm, whereas that synthesised in bulk solution was 9 ± 3 nm (Figure 1.19 B).

![Figure 1.19](image)

Figure 1.19 (A) Droplet-based microfluidic device for the synthesis of magnetic iron oxide nanoparticles, where fusion of droplets was achieved by electrocoalescence. (B) TEM micrograph showing 4 ± 1 nm iron oxide nanoparticles. The inset shows the monocrystalline nature of the nanoparticles. Image taken from reference [108].

In another example, titanium oxide was produced in a single-phase continuous microfluidic reactor. Microfluidic synthesis yielded nanorods with high anisotropy and high surface-to-volume ratios. Moreover, the reaction time was reduced from 90 minutes under bulk conditions to 80 seconds in the microfluidic system. Moreover, Weng et al. synthesised hollow Fe/Ga-based oxide nanospheres in a microfluidic system integrating a micro-mixer, micro-condenser, micro-valves, micro-heater and micro-temperature sensor and obtained nanoparticles with a diameter of 157 ± 26 nm. A range of other metal oxide nanoparticles
have been synthesised in both continuous and segmented flow microfluidic systems and are described in a review by Marre et al.[111]

1.4.5.4 Microfluidic Synthesis of CaCO$_3$

To date, the only microfluidic method reported for the synthesis of CaCO$_3$ involved the continuous flow mixing of reagents at a T-junction in a microfluidic device.[112] On-line characterisation of the crystals was achieved by Raman spectroscopy. However, the unavoidable residence time distributions associated with single-phase continuous flow devices generated large size distributions of the resulting crystals of CaCO$_3$. Subsequently, the same authors used single-phase microfluidic flows to demonstrate the influence of extrapallial (EP) 28 kDa protein on the morphology, structure and polymorph of CaCO$_3$.[113] They obtained novel lemon-shaped hollow vaterite particles when the EP protein was initially present only in the Ca$^{2+}$ solution (rather than in CO$_2^-$ solution). The authors concluded that in order to obtain the hollow vaterite, a stable EP protein and Ca$^{2+}$ concentration gradient must co-exist. This is not possible in bulk systems where rapid mixing is driven by a turbulent flow, with only rhombohedral calcite being formed.

1.5 Summary of Thesis

As noted, droplet-based microfluidic systems are becoming an increasingly attractive tool for a variety of synthetic applications. They are a specially interesting alternative to the synthesis of nanoparticles in bulk systems because it is possible to maintain a highly controlled reaction environment, where both nucleation and growth can be controlled or varied.

The primary aim of the work described herein is to create a system where the crystallisation of calcium carbonate can be studied in detail and crystals with the required properties synthesised at will. A thermodynamically stable polymorph is first synthesised using a droplet-based microfluidic method at varying reagent concentrations. The properties of the obtained crystals are then compared with the crystals synthesised in both a single-flow
microfluidic system and bulk. In the second part of the thesis, the growth of amorphous particles is studied thoroughly at different residence times and compared with those synthesised using conventional bulk methods. In the third part of the thesis the influence of polymers on the growth of calcium carbonate is investigated. All studies are aimed at establishing a droplet-based microfluidic platform for the study of crystallisation of inorganic materials and subsequent control of their properties.
Chapter 2

Methods and Materials

The fabrication and operation of the microfluidic devices used in this thesis are described, together with details of all chemical and analytical procedures. The characterisation and visualisation of droplets within segmented flows is also presented together with an assessment of the conditions required to obtain uniformly sized and spaced droplets.
2.1 Microfluidic Device Fabrication

All microfluidic devices used in the current studies were fabricated in polydimethylsiloxane (PDMS). The required design was first printed onto a photomask, which was then used to fabricate a master. This master was then used to cast final devices in PDMS. Device fabrication was carried out in a Class 10000 cleanroom in the Chemistry Department at Imperial College London.

2.1.1 Fabrication of SU-8 Master

A high-resolution photomask design for a microchannel network was created using CAD software (AutoCAD 2005, Autodesk, California, USA). Designs were then transferred to a high-resolution acetate film (JD Photo-Tools, Oldham, UK). Final designs had a minimum feature size of 10 µm. A thin layer of SU8-50 negative photoresist (MicroChem Corporation, Newton, MA, USA) was deposited onto the surface of a silicon wafer (IBD Technologies Ltd, UK) via spin coating (Figure 2.1). Spin coating was carried out in two steps; initially photoresist was added to the spinning wafer at 500 rpm for 10 seconds. This allows the photoresist to cover the whole surface of the wafer. In a second step, the wafer was spun at 1000 rpm for 30 seconds, to obtain a final resist layer thickness of 100 nm. The coated wafer was then subjected to a soft bake (Figure 2.1). This involved pre-baking at 65 ºC for 10 minutes to evaporate the solvent in which the resist polymer was dissolved. The temperature was then increased to 95ºC and the wafer baked for an additional 30 minutes (Figure 2.1). The photomask was then placed on top of the photoresist-covered wafer and exposed to UV radiation (OIA, San Jose, CA, USA) (Figure 2.1). The exposure time was defined by the SU8 thickness but was typically around 30 seconds (equivalent to 300 mJ/cm²). A two-step post exposure bake was performed to selectively cross link the exposed SU8 film. Initially the wafer was heated at 65°C for 1 minute. Then the temperature was increased to 90°C and the wafer heated for a further 10 minutes. The wafer was then immersed in a SU8 developer solution (Microdeposit EC-Solvent, Chestech Ltd, UK) to remove unexposed SU8. After development, the patterned wafer was washed with iso-propanol and dried with nitrogen.
Figure 2.1 Processes involved in PDMS device fabrication. 1) Spin coating of an Si wafer with SU8, 2) exposure of SU8 to UV radiation, 3) development of unexposed SU8, 4) casting of PDMS and 5) and 6) assembly of device.
2.1.2 PDMS Moulding

A SYLGARD 184 Silicone Elastomer Kit (Dow Corning Ltd, UK) was used to form PDMS devices. The tetra(trimethylsiloxy)silane base and curing agent (tetramethyltetravinylcyclotetrasiloxane), were mixed together in a 10:1 ratio to yield a total mass of approximately 40 g. After vigorous stirring with a plastic rod, the mixture was degassed in a dessicator for 10 minutes. The mixture was then poured over the SU8 master and cured at 65ºC on a hot plate for 1 hour. Once cured, the PDMS was detached from the master and diced. Input and output holes were created in the device using a 1 mm biopsy punch (Kai Medical, Japan). The bottom layer of the microfluidic device was then prepared by curing 10 g of PDMS mix in a square Petri dish for 30 minutes to generate a partially cured surface. The two layers were then joined together and cured in an oven at 65ºC overnight.

2.1.3 Microfluidic Device Assembly

The resulting microfluidic device contained three inlets, with all microchannels having a uniform cross section of 100 µm (width) x 50 µm (height). The wider section at the end of the reaction channel has a cross section of 300 µm (width) x 50 µm (height) and is used to interface with outlet tubing. Mini filters present in the inlet channels were used to trap large particulates, thus reducing the possibility of channel blockage. Polyethylene tubing (380 µm I.D. and 1.09 mm O.D., Harvard Apparatus Ltd, UK) was used to deliver reagents and oil into the device. The use of tubing with a slightly larger diameter than that of the access holes ensures a water-tight seal. Poly(tetrafluoroethylene) (PTFE) tubing (Cole Palmer, Hanwell, UK) of 100 µm I.D. and 300 µm O.D. was inserted carefully into the outlet reservoir to both extend the reaction channel and allow efficient collection of products.
2.2 Droplet Microfluidic Device Operation

Aqueous reagents and the oil phase were delivered into the microfluidic device using precision syringe pumps (PHD 2000, Harvard Apparatus, Holliston, MA, USA) at volumetric flow rates ranging from 1 to 10 µl/min. A high-speed camera (Phantom®, v 649, Vision Research, Bedford, UK) was used to image droplets using a 50 µs exposure time and a frame rate of 1000 frames per second (fps). An annotated photograph of the setup used for all microfluidic experiments described in this thesis is shown in Figure 2.3.
Figure 2.3 Annotated photograph of the microfluidic setup used for all experiments, showing the device on a microscope stage, syringe pumps and the tubing used to deliver reagents and oil to the device.

2.2.1 Droplet-Based Microfluidic Device Characterisation

Preliminary studies of droplet formation and characterisation were carried out using a flow focusing microfluidic device. An aqueous phase of deionised water and a carrier phase of Mineral oil (Sigma Aldrich) containing 2.5 % ABIL EM90 surfactant were used for all experiments. An inverted microscope (Olympus UK Ltd, UK) equipped with a high-speed camera (Phantom v 649, Vision Research Ltd, UK) was used to image the microfluidic device during operation. An average droplet length was calculated from measurement of at least 100 droplets using ImageJ (Version 1.42, National Institute of Health). Droplet volume was calculated assuming a cuboid volume, and channel dimensions of 100 μm (width) and 50 μm (height).
Droplet size was controlled by varying the relative flow rates of the two phases and quantified as a function of the ratio of the aqueous (dispersed phase) flow rate to the oil (continuous phase) flow rate \( \frac{Q_a}{Q_o} \). To generate large droplets, the flow rate of the continuous phase was decreased relative to that of the dispersed phase. Figure 2.4 A presents images of droplets generated at different \( \frac{Q_a}{Q_o} \) ratios whilst maintaining a constant total flow rate. The variation in droplet volume as a function of total flow rate is presented in Figure 2.4 C, and shows that the average droplet volume is independent of the total flow rate when \( \frac{Q_a}{Q_o} \) is fixed. However, \( \frac{Q_a}{Q_o} \) determines droplet size, and consequently droplet volume. Increasing \( \frac{Q_a}{Q_o} \) leads to larger droplets, as seen in Figure 2.4 A. Accordingly, it is demonstrated that there exists a strong dependence of droplet volume on \( \frac{Q_a}{Q_o} \) and not on the total flow rate. Figure 2.4 B shows the variation of droplet period as a function of the total flow rate. Similarly, it can be seen that the droplet period varies with \( \frac{Q_a}{Q_o} \) and not with the total flow rate.

![Image of droplets](image1.png)

Figure 2.4 (A) Images of droplets formed at different \( \frac{Q_a}{Q_o} \) ratios. Droplets were generated using a total flow rate of 20 \( \mu \)l/min. (B) Variation of droplet period as a function of the total flow rate. (C) Variation of droplet volume as a function of the total flow rate. The droplet volume remains relatively constant when \( \frac{Q_a}{Q_o} \) is constant, but the flow rate is changed.
2.2.2 Long Term Device Operation

One of the most challenging issues faced when carrying out reactions in microfluidic devices is the unstable flow of the droplets. This can lead to a number of outcomes, such as varying droplet volumes (and thus varying amount of reagents in each droplet), varying distance between droplets (a smaller distance between droplets can result droplets merging), and occasional inhibition of the flow and rupture of the device (due to pressure build up). Figure 2.5 shows a comparison of stable and unstable droplet generation. Video 2.1 (DVD provided with this thesis) illustrates stable droplet generation in a microfluidic device, where droplets are of a uniform size and spacing. In order to achieve a stable droplet flow in the microfluidic device used in this thesis, the following guidelines, developed empirically were followed:

- The flow rates for each inlet flow are never less than 0.3 µl/min. At lower flow rates, droplet generation is unstable.
- The flow rates for each inlet flow are never more than 15 µl/min, since higher flow rates shorten the lifetime of the microfluidic device due to pressure build-up.
- For high \(\frac{Q_a}{Q_o}\) ratios (> 1.25), the flow rate of the oil is much lower than that of the aqueous phase. This results in the aqueous stream occasionally entering the oil inlet and causing wetting problems. Wetting occurs when the oil phase is not able to completely wet the surface of the channel, resulting in a decreased oil flow. Accordingly \(\frac{Q_a}{Q_o}\) ratios lower than 1.25 were used throughout.
- For low \(\frac{Q_a}{Q_o}\) ratios (< 0.2), the flow rate of the aqueous phase is much lower than that of the oil phase, resulting in occasional back-flow of oil. This causes a laminar flow of the reagents and fouling of the channel walls. Accordingly \(\frac{Q_a}{Q_o}\) ratios were kept between 0.2 and 1.25.
Another important and unusual feature of the microfluidic devices used in this thesis is the extension of the reaction channel with the use of PTFE tubing. It is essential that the droplets entering the tubing do not break up or merge. Figure 2.6 shows images of droplets flowing in both a microchannel and PTFE tubing. It is clear that once transferred into the tubing, droplets are still of uniform size and with a constant distance between them. Video 2.2 (DVD provided with this thesis) shows the smooth transition of droplets from the device into the tubing. In order to achieve such a transition, the tubing must be cut at a right angle (to prevent droplet breakup) and inserted into the output of the PDMS device carefully to avoid rupturing of the channel. Rupturing of the channel results in both leakage and a shortening of the device lifetime.
2.3 Off-line Analysis of Droplet Contents

In this thesis reaction products were analysed by Raman spectroscopy, scanning electron microscopy (SEM) and transmission electron microscopy (TEM). Brief details of the instrumentation and methods are now given.

2.3.1 Raman Spectroscopy

Raman spectroscopy was used for CaCO₃ polymorph analysis. Spectra were obtained using either a LabRAM HR800 Raman spectrometer (Jobin Yvon, Stanmore, UK) with a 632.8 nm excitation source or a Renishaw 2000 inVia-Raman microscope (Renishaw, Wotton-under-Edge, UK), operating at 785 nm. An average of three spectra were used for all analyses.

2.3.2 Scanning Electron Microscopy

SEM was used for particles imaging and size distribution analysis. Precipitates were supported on track-etch membranes (Millipore, 0.2 μm pore size), sputter coated with gold/chromium to prevent charge accumulation and examined on a JEOL5610 scanning electron microscope using an acceleration voltage of 15 kV and a working distance of 5-10 mm. High-resolution images were acquired using a LEO 1525 FEG-SEM at a 5 kV operating voltage and a 6-7 mm working distance.

2.3.3 Transmission Electron Microscopy

TEM was used for high magnification morphological analysis and crystallinity analysis. Precipitates were deposited onto a 100 x 100 carbon thin film on a Cu mesh (Agar Scientific) and imaged using a JEOL 2000FX TEM at a 200 kV gun voltage and exposure times of between 3 and 10 s. High resolution TEM images were obtained using a FEI Tecnai TF20 FEGTEM at 200 kV gun voltage and an exposure time of 3-10 s.
2.3.4 Analytical Software

Crystal size distributions were determined using ImageJ software (version 1.42, National Institute of Health). For each image, the image scale was set before measuring the size of each individual particle. At least 100 particles were analysed in each experiment.
Chapter 3

Precipitation of Calcium Carbonate

Studies reported in this chapter assess three different platforms for the synthesis of calcium carbonate crystals: droplet-based microfluidics, continuous flow microfluidics and conventional bulk methods. For each method, particles were synthesized under a range of experimental conditions, collected and then analysed using Raman spectroscopy (to determine polymorphism) and SEM (to assess particle size and population size distribution). The operational performance of each platform is compared and contrasted.
3.1 Introduction

Calcium carbonate is one of the most studied inorganic crystals due to its industrial applications, its role in the environment and in geology, and its importance as a biomineral. Because it can precipitate into a number of polymorphs under ambient conditions, it is recognized to be a valuable model system for understanding crystallisation of low-solubility minerals. In practice, typical biomimetic experiments aimed at studying nucleation and crystallization of biominerals are performed in bulk (macroscale) environments. Under these conditions, it is difficult to produce crystals of controlled size, shape and crystal structure, since both kinetic and thermodynamic control of the reaction process is required. Accordingly, almost all traditional approaches involve the use of wet-chemical methods, polymer templates or soluble additives and require multiple purification steps post-reaction to obtain crystals of desired polymorph and structure.

To date, control of the morphological and structural complexity of calcium carbonate crystals has been achieved using a number of different methods. These include the use of soluble additives (anionic surfactants, block copolymers, biopolymers, the use of templates, the addition of metals as impurities and the incorporation of gels, vesicles, micelles or microemulsions, as well as variation and control of precipitation conditions such as temperature and pH. For instance, Chen et al. showed that the structure and morphology of CaCO₃ crystals can be controlled by simply varying the reaction temperature, without the need for additives. In these experiments vaterite aggregates were formed at temperatures between 30 and 40°C, a mixture of vaterite, aragonite and calcite was formed at temperatures between at 50 and 70°C and aragonite only formed at 80°C. In addition, Han and co-workers showed that synthesis high pH lead to an increase in supersaturation (resulting from the conversion of HCO₃⁻ to CO₃²⁻) and the formation of more nuclei. This pH change promotes the growth of vaterite nuclei and inhibits the transformation of vaterite into calcite.

Several model systems have also been established to study the crystallisation of CaCO₃ in confined conditions, including track-etched membranes and droplet arrays. Of particular relevance to the work described herein is the bottom-up synthesis of single calcite...
crystals on a micropatterned substrate by Aizenberg and co-workers. Here, amorphous calcium carbonate was precipitated on micropatterned templates and resulted in the creation of millimeter-sized single calcite crystals with controlled crystallographic orientation (Figure 3.1).

![Diagram](image)

Figure 3.1 (A) Templates for quasi-two dimensional (q2D) micropatterns for mineral deposition where formation of photoresist micropatterns was carried out by standard photolithographic procedures. (B) Deposition of calcium carbonate was carried out by placing the q2D substrate in a CaCl$_2$ solution in a closed desiccator containing (NH$_4$)$_2$CO$_3$ powder. (C) SEM micrograph of micropatterned single crystals on calcite. The inset shows a TEM diffraction pattern corresponding to a single crystal of calcite. Image taken from reference [18].
Confinement of reagents in droplet-based microfluidic systems offers a more controlled way of precipitating inorganic materials. To date, the only microfluidic approach reported for the synthesis of calcium carbonate crystals involved the continuous flow mixing of reagents at a T-junction in a microfluidic device with product characterisation via in situ Raman spectroscopy.[112] This simple proof-of-principle study demonstrated facile crystal formation. However, the unavoidable residence time distributions associated with continuous-flow reactors reduced control over reaction times and generated large size distributions of the resulting crystals. This, in turn, limits the synthetic influence over crystal polymorph.

The use of droplet-based microfluidic systems to investigate the precipitation of calcium carbonate under highly controlled conditions provides a unique opportunity for investigating mineral precipitation in small volumes and under well-defined experimental conditions. Moreover, the use of pL-volume droplets as environments in which to perform precipitation has special relevance to weathering and biomineralisation, which invariably occur in restricted volumes rather than in bulk solution.[129,130]

3.2 Experimental

3.2.1 Segmented Flow Microfluidic Synthesis of CaCO₃

Equimolar solutions of calcium chloride and sodium carbonate in 1 ml syringes (4, 5, 8, 10 and 12 mM) were delivered through the two inlets of the microfluidic reactor at volumetric flow rates of 1 µl/min (Figure 3.2). To generate a segmented flow consisting of aqueous droplets surrounded by a continuous oil phase, the combined aqueous stream was delivered to a flow focusing junction where it meets two orthogonal flows of FC-40 oil (3M, Bracknell, UK) containing 2.5 % Raindance surfactant (Raindance Technologies, Lexington, MA) at 3 µl/min (Figure 3.2). The formed droplets are then manoeuvred along a reaction channel for 30 s (40 cm). Reaction products were collected at the outlet of the PTFE tubing (Cole Palmer, Hanwell, UK) and directly transferred onto a 0.2 μm pore size track-etch membrane.
(Millipore, London, UK). Product was then washed with ethanol under suction and dried at room temperature.

**Figure 3.2** Schematic of the microfluidic system used for droplet-based microfluidic synthesis of calcium carbonate. Droplets are formed by flow focusing two aqueous streams of reagents with FC-40 oil.

### 3.2.2 Continuous Flow Microfluidic Synthesis of CaCO₃

Equimolar solutions of calcium chloride and sodium carbonate in 1 ml syringes (4, 5, 8, 10 and 12 mM) were delivered into the microfluidic reactor at a volumetric flow rate of 1 µl/min. In this case, the solutions were allowed to flow parallel to each other through both the microfluidic channel and PTFE tubing (Figure 3.3). The channel dimensions were the same as those in the segmented flow microfluidic device described in Chapter 2, i.e a channel depth of 50 µm and a channel width of 100 µm. Products were collected on a track-etch membrane, washed with ethanol under suction and dried at room temperature.
3.2.3 Bulk Synthesis of CaCO$_3$

100 ml of equimolar solutions of aqueous calcium chloride and sodium carbonate (4, 5, 8, 10 and 12 mM) were mixed in a 200 mL glass beaker. After a defined time period, the precipitated mixture was filtered through a track-etch membrane, washed with ethanol and dried at room temperature.

3.2.4 Analysis of Reaction Products

Particles collected on track-etch membranes were first analyzed by Raman microscopy to identify the predominant crystal polymorphs present. Spectra were obtained either on a LabRAM HR800 Raman spectrometer (Jobin Yvon, Stanmore, UK) using a 632.8 nm excitation source or a Renishaw 2000 inVia-Raman microscope (Renishaw, Wotton-under-
Calcite is easily characterized by observation of vibrational bands at 711 cm\(^{-1}\) (\(v_4\)) and 1085 cm\(^{-1}\) (\(v_1\)), whereas vaterite is identified by a doublet peak centered at 1088 cm\(^{-1}\) (\(v_1\)).[131] Subsequent to Raman microscopy, samples were sputter coated with gold and imaged using scanning electron microscopy (SEM). Particle size distributions were determined directly from SEM images using ImageJ, according to the procedure described in Chapter 2. The mean particle size and population standard deviation were calculated from an analysis of a minimum of 100 particles.

3.3 Results

3.3.1 Segmented Flow Microfluidic Synthesis

As stated, calcium carbonate was precipitated by combining equal volumes of equimolar solutions of CaCl\(_2\) and Na\(_2\)CO\(_3\) in 200 pL droplets. Concentrations of both reagents ranged from 4 to 12 mM. Attempts to precipitate at concentrations below 4 mM produced an insufficient number of particles for further analysis. When precipitating at concentrations above 12 mM, fouling of the microfluidic channels resulted in shorter running times for each experiment. In situations where both vaterite and calcite polymorphs were precipitated, only calcite crystals were analysed by SEM, since vaterite is metastable and eventually transforms into calcite.[54]

SEM images of precipitates collected from 4 mM solutions consistently reported a mixture of rhombohedral and truncated rhombohedral crystals between 1 and 3 \(\mu\)m in size (Figure 3.4 A and C), having a mean size of 2 \(\mu\)m and a population standard deviation of 0.24 \(\mu\)m. Raman spectra (Figure 3.4 B) confirmed the presence of the calcite polymorph through the observation of bands at 711 cm\(^{-1}\) (\(v_4\)), 1085 cm\(^{-1}\) (\(v\)), and 281 cm\(^{-1}\) (lattice mode vibrations).
Figure 3.4  (A) SEM micrograph of CaCO$_3$ particles precipitated in 250 pL droplets from 4 mM Na$_2$CO$_3$ and CaCl$_2$ solutions. The inset shows a high magnification image of selected particles. (B) The corresponding Raman spectrum of a single particle confirming the calcite polymorph. (C) Size distribution of the precipitated calcite particles.

Analysis of product precipitated from 5 mM solutions, indicated the presence of truncated rhombohedral, rhombohedral and spherical crystals between 1 and 4 µm in size (Figure 3.5 A and D). Raman spectroscopic measurements showed that the rhombohedral crystals were calcite through observation of bands at 711 cm$^{-1}$ ($\nu_4$), 1085 cm$^{-1}$ ($\nu$), and 286 cm$^{-1}$ (Figure 3.5 B). Analysis of spherical crystals showed a doublet peak at 1088 cm$^{-1}$ associated with the vaterite polymorph (Figure 3.5 C). The mean size and population standard deviation of calcite crystals were calculated to be 2.4 and 0.3 µm respectively.
Precipitates collected after reaction of 8 mM solutions were spherical in nature and between 3 and 7 µm in size (Figure 3.6 A and C). High resolution SEM revealed that the spherical crystals were actually aggregates of 40 nm primary nanoparticles (Figure 3.6 A). Raman spectroscopy confirmed the existence of the vaterite polymorph by observation of the doublet peak at 1088 cm\(^{-1}\) (Figure 3.6 B). The mean particle size and population standard deviation were calculated to be 5.0 and 0.4 µm respectively.
Figure 3.6  (A) SEM micrograph of CaCO\textsubscript{3} particles precipitated in 250 pL droplets from 8 mM Na\textsubscript{2}CO\textsubscript{3} and CaCl\textsubscript{2} solutions. The inset shows a high magnification image of selected particles. (B) The corresponding Raman spectrum of a single particle confirming the vaterite polymorph. (C) Size distribution of the precipitated vaterite particles.

For the reaction of 10 mM solutions, SEM images revealed a mixture of rhombohedral, truncated rhombohedral and spherical crystals (Figure 3.7 A). Rhombohedral crystals, were confirmed by Raman spectroscopy (Figure 3.7 B) to be the calcite polymorph through the observation of bands at 711 cm\textsuperscript{-1} (\nu\textsubscript{4}), 1085 cm\textsuperscript{-1} (\nu), and 286 cm\textsuperscript{-1}, and were between 1 and 5 \textmu m in size (Figure 3.7 D). The observation a doublet peak at 1088 cm\textsuperscript{-1} confirmed vaterite polymorph (Figure 3.7 C). The mean size and population standard deviation were calculated to be 2.2 and 0.4 \textmu m respectively.
Finally, a mixture of calcite and vaterite polymorphs (confirmed by Raman spectroscopy) was precipitated from 12 mM solutions (Figure 3.8 A). Calcite crystals were between 1 and 4 µm in size (Figure 3.8 D) and exhibited Raman bands at 711 cm\(^{-1}\) (\(\nu_4\)), 1085 cm\(^{-1}\) (\(\nu\)), and 280 cm\(^{-1}\) (lattice mode vibrations) (Figure 3.8 B). Vaterite crystals were confirmed by a doublet at 1088 cm\(^{-1}\) (Figure 3.8 C). The mean size and population standard deviation of calcite crystals were calculated to be 2.8 and 0.3 µm respectively.
Figure 3.8  (A) SEM micrograph of CaCO$_3$ particles precipitated in 250 pL droplets from 12 mM Na$_2$CO$_3$ and CaCl$_2$ solutions. (B) The corresponding Raman spectrum of a single rhombohedral particle confirming the calcite polymorph (C) The corresponding Raman spectrum of a single spherical particle confirming the vaterite polymorph. (D) Size distribution of the precipitated calcite particles.

The mean precipitate size and population standard deviation for each of the above reactions is summarized in Table 3.1.

<table>
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<tr>
<th>Concentration (mM)</th>
<th>Size distribution (µm)</th>
<th>Mean size (µm)</th>
<th>Standard Deviation (µm)</th>
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<tr>
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<td>0.3</td>
</tr>
<tr>
<td>8</td>
<td>2.7-6.4</td>
<td>5.0</td>
<td>0.4</td>
</tr>
<tr>
<td>10</td>
<td>1.0-4.6</td>
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</tr>
<tr>
<td>12</td>
<td>1.5-3.9</td>
<td>2.8</td>
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</tr>
</tbody>
</table>

Table 3.1  Calculated size distribution, mean size and standard deviation for calcite precipitated from (4, 5, 10 and 12 mM) Na$_2$CO$_3$ and CaCl$_2$ solutions and vaterite precipitated from 8 mM Na$_2$CO$_3$ and CaCl$_2$ solutions in 250 pL droplets.
3.3.2 Continuous Flow Microfluidic Synthesis

In the continuous flow method, fluids move under laminar flow.[76] In a laminar flow regime, fluids do not mix convectively when they come together but flow in parallel and only mix via diffusion across the fluidic interface.[18] Accordingly, variation of the channel dimensions and the volumetric flow rates of the streams provides a direct method of controlling the rate of mixing. In order to evaluate mixing efficiency in the continuous flow regime, the Fourier number was calculated. For a residence time of 40 s, a diffusion length of $10^{-4}$ m and an average diffusion coefficient of $10^{-9}$ m$^2$/s the Fourier number (Fo) is 4. Hence complete mixing of reagents is achieved during passage through the device.[18] To allow direct comparison with the segmented flow microfluidic studies presented in Section 3.3.1, calcium carbonate was precipitated from the same range of reagent concentrations (4, 5, 8, 10 and 12 mM).

For all reagent concentrations studied, a mixture of rhombohedral, truncated rhombohedral, tetrahedral and spherical crystals was obtained. Raman spectroscopy (Figure 3.10) confirmed the rhombohedral, truncated rhombohedral, tetrahedral crystals to be calcite and the spherical crystals to be vaterite. Figure 3.9 shows images of the CaCO$_3$ particles precipitated in the continuous flow microfluidic device from (A) 4 mM, (B) 5 mM, (C) 8 mM, (D) 10 mM and (E) 12 mM solutions respectively.

Table 3.2 reports the respective particle size distribution, mean size and population standard deviation.
Figure 3.9 Photomicrographs of CaCO$_3$ particles precipitated in a continuous flow microfluidic device by combination of CaCl$_2$ and Na$_2$CO$_3$ solutions at concentrations of (A) 4 mM, (B) 5 mM, (C) 8 mM, (D) 10 mM and (E) 12 mM. Scale bar: 50µm.
Figure 3.10 (A) A typical Raman spectrum of a single rhombohedral particle precipitated from 4, 5, 8, 10 and 12 mM Na$_2$CO$_3$ and CaCl$_2$ solutions in a continuous flow regime, confirming the calcite polymorph. (B) A typical Raman spectrum of a single spherical particle precipitated from 4, 5, 8, 10 and 12 mM Na$_2$CO$_3$ and CaCl$_2$ solutions in a continuous flow regime, confirming the vaterite polymorph.

<table>
<thead>
<tr>
<th>Concentration (mM)</th>
<th>Size distribution (µm)</th>
<th>Mean size (µm)</th>
<th>Standard Deviation (µm)</th>
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<td>0.9</td>
</tr>
<tr>
<td>5</td>
<td>5.8-9.5</td>
<td>7.7</td>
<td>0.3</td>
</tr>
<tr>
<td>8</td>
<td>5.1-10.8</td>
<td>8.5</td>
<td>0.6</td>
</tr>
<tr>
<td>10</td>
<td>3.1-6.4</td>
<td>4.6</td>
<td>0.3</td>
</tr>
<tr>
<td>12</td>
<td>3.2-7.3</td>
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</tr>
</tbody>
</table>

Table 3.2 Size distribution, mean size and standard deviation for calcite polymorph precipitated in continuous flow microfluidic device from reagent concentrations of 4-12 mM.

3.3.3 Bulk Synthesis of CaCO$_3$

Precipitation of calcium carbonate in bulk was used as a control for all microfluidic experiments. Calcium carbonate was precipitated using a range of reagent concentrations (between 4 and 12 mM). For all conditions, a mixture of rhombohedral and spherical particles was obtained, with Raman spectroscopic analysis revealing the presence of both calcite and
vaterite polymorphs.

Figure 3.11 A shows precipitate collected from reaction of 4 mM solutions. Single crystal Raman spectra confirmed the presence of both calcite and vaterite polymorphs (Figure 3.11 B and C) with bands at $711\text{cm}^{-1}$ ($\nu_4$), $1085\text{ cm}^{-1}$ ($\nu$), and $286\text{ cm}^{-1}$ reporting the calcite polymorph and a doublet at $1088\text{ cm}^{-1}$ reporting the vaterite polymorph. In addition, Figure 3.11 D demonstrates that calcite crystals in the product mixture range between 4.4 and 17.7 $\mu$m in size, with a mean size and population standard deviation of 8.8 and 1.5 $\mu$m respectively (Table 3.3).

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Figure 3.11  (A) SEM micrograph of CaCO$_3$ particles precipitated in bulk from 4 mM Na$_2$CO$_3$ and CaCl$_2$ solutions. (B) Corresponding Raman spectrum of single rhombohedral particle confirming the presence of the calcite polymorph. (C) Corresponding Raman spectrum of single spherical particle confirming the presence of the vaterite polymorph. (D) Size distribution of the precipitated calcite particles.
Particles collected from the reaction of 5 mM solutions (Figure 3.12 A) were confirmed by Raman spectroscopic measurements to represent both calcite and vaterite polymorphs (Figure 3.12 B and C). Moreover, Figure 3.12 D demonstrates that crystals with sizes between 2.7 and 12.9 µm were produced, with a mean size and population standard deviation of 8.1 µm and 1.1 µm respectively (Table 3.3).

![SEM micrograph of CaCO₃ particles precipitated in bulk from 5 mM Na₂CO₃ and CaCl₂ solutions. (B) Corresponding Raman spectrum of a single rhombohedral particle confirming the presence of the calcite polymorph. (C) Corresponding Raman spectrum of a single spherical particle confirming the presence of the vaterite polymorph. (D) Size distribution of the precipitated calcite particles.](image)

SEM micrographs of particles precipitated from 8 mM solutions (Figure 3.13 A) reveal the presence of calcite and vaterite as well as submicron particles which have not yet re-precipitated to form calcite or vaterite polymorphs, and are presumably amorphous calcium carbonate (ACC).[65] Raman spectroscopic analysis of the product revealed the presence of
both calcite and vaterite polymorphs. Rhombohedral crystals, were confirmed by Raman spectroscopy (Figure 3.13 B) to be the calcite polymorph through the observation of bands at 711 cm\(^{-1}\) (\(\nu_4\)), 1085 cm\(^{-1}\) (\(\nu\)), and 281 cm\(^{-1}\). Vaterite crystals were confirmed by a doublet at 1088 cm\(^{-1}\). Interestingly, there is an absence of ACC in regions immediately adjacent to calcite and vaterite crystals. This phenomenon has been previously observed and described as “localized depletion” by Aizenberg et al.[128] Under these conditions, calcite crystals varied between 1.7 and 11.5 µm in size (Figure 3.13 D), with a mean size and population standard deviation of 6.6 µm and 1 µm respectively (Table 3.3).

![Figure 3.13](image_url) (A) SEM micrograph of CaCO\(_3\) particles precipitated in bulk from 8 mM Na\(_2\)CO\(_3\) and CaCl\(_2\) solutions. (B) Corresponding Raman spectrum of a single rhombohedral particle confirming the presence of the calcite polymorph. (C) Corresponding Raman spectrum of a single spherical particle confirming the presence of the vaterite polymorph. D) Size distribution of the precipitated calcite particles.

For precipitates collected from 10 mM solutions, similar results to those obtained at 8 mM were observed. Now the number of submicron particles exceeds the number of calcite and
vaterite particles formed (Figure 3.14 A). The size distribution (Figure 3.14 D), average size and population standard deviation of the calcite crystals were 3.7-14.0 µm, 8.5 µm and 1.4 µm respectively (Table 3.3). Raman spectra (Figure 3.14 B) confirmed the presence of the calcite polymorph through the observation of bands at 711 cm\(^{-1}\) (\(\nu_4\)), 1085 cm\(^{-1}\) (\(\nu\)), and 281 cm\(^{-1}\) and vaterite through a doublet at 1088 cm\(^{-1}\).

![Figure 3.14](image)

Figure 3.14  (A) SEM micrograph of CaCO\(_3\) particles precipitated in bulk from 10 mM Na\(_2\)CO\(_3\) and CaCl\(_2\) solutions. (B) Corresponding Raman spectrum of a single rhombohedral particle confirming the presence of the calcite polymorph. (C) Corresponding Raman spectrum of a single spherical particle confirming the presence of the vaterite polymorph. (D) Size distribution of the precipitated calcite particles.

Calcite and vaterite particles precipitated from 12 mM solutions were found to be completely embedded in a crust of densely formed submicron particles (Figure 3.15 A). The particle size distribution (Figure 3.15 D), average size and population standard deviation of the calcite crystals were 5.9-14.5 µm, 10.8 µm and 0.9 µm respectively (Table 3.3). Rhombohedral crystals were identified as calcite by Raman spectroscopy through the presence of bands at 711 cm\(^{-1}\) (\(\nu_4\)), 1085 cm\(^{-1}\) (\(\nu\)), and 281 cm\(^{-1}\) (Figure 3.15 B). Spherical crystals were identified as vaterite through the observation of a doublet at 1088 cm\(^{-1}\) (Figure 3.15 C)
Figure 3.15  (A) SEM micrograph of CaCO$_3$ particles precipitated in bulk from 12 mM Na$_2$CO$_3$ and CaCl$_2$ solutions. (B) Corresponding Raman spectrum of a single rhombohedral particle confirming the presence of the calcite polymorph. (C) Corresponding Raman spectrum of a single spherical particle confirming the presence of the vaterite polymorph. (D) Size distribution of the precipitated calcite particles.

<table>
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<td>12</td>
<td>5.9-14.5</td>
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</table>

Table 3.3  Size distribution, mean size and standard deviation for calcite polymorph particles precipitated in bulk from reagent concentrations between 4 and 12 mM.
3.4 Discussion

Monodisperse and impurity-free droplets provide well-defined environments in which to perform the CaCO$_3$ crystallization reaction, where nucleation occurs homogenously or at the aqueous/oil interface. It is likely that crystal nucleation in each droplet occurs over a short period of time, followed by crystals growing in competition with each other.[94] As a result, precipitated crystals are of near identical size, especially when compared to single flow and bulk syntheses. For example, for 4 mM reagents the particle size distributions for droplet, single flow and bulk syntheses are 1.0-3.1 µm, 3.3-11.5 µm and 4.4-17.7 µm respectively with standard deviations of 0.2, 0.9 and 1.5 µm respectively.

The smaller average size of calcium carbonate crystals precipitated in the segmented flow regime (2-3 µm) is due to the fact that the 250 pL droplets contain a limited pool of reagents, which is rapidly consumed by growing nuclei, resulting in the termination of crystal growth. As a result, varying the concentration of initial reagents should not have a pronounced effect on the size of the calcite crystals. Indeed, for each concentration studied, calcite particles were precipitated with a mean size of between 2 and 3 µm.

Control over crystal polymorph was also observed in droplets (calcite alone was precipitated from 4 mM solutions and vaterite alone from 8 mM solutions). This control is attributed to the lack of impurities and nucleation surfaces within the droplet, which promotes homogeneous nucleation. The surfactant employed in the carrier oil is non-ionic and is therefore unlikely to promote nucleation.[88] The fact that only vaterite crystals are precipitated from 8 mM solutions is unusual, since it is typically very difficult to obtain pure vaterite in the absence of additives.[132] Previously, additives including certain polymers,[133,134] small molecules[135] and ammonium ions,[73,127,136] have been employed to produce pure vaterite. However, at low supersaturations, there are only a few reports that pure vaterite can be precipitated under specific reaction conditions. For example, vaterite alone was precipitated when the concentration of [Ca$^{2+}$] and [CO$_3^{2-}$] was 2.5 mM and pH was between 9.3 to 9.9.[132] At high supersaturations, vaterite forms as a precursor to calcite, but its transformation to calcite is rapid.[137] In contrast, only vaterite precipitates without calcite seeds when the ion activity product of the initial supersaturated solution is
lower than the solubility of amorphous calcium carbonate (ACC).[138]

In a single-phase, continuous flow regime, the walls of the microchannel impart shear forces on the fluid, thus establishing a parabolic velocity profile under an applied hydrodynamic pressure.[18] Accordingly, fluid at the channel walls moves at a lower velocity compared to fluid at the centre of the channel. Therefore product collected from such a reactor will consist of elements that have spent varying amounts of time within the reactor, and will generate a wider size distribution of the formed particles compared to the segmented flow regime where droplets move at a fixed linear velocity. For 4 mM solutions, crystals between 1.0 and 3.1 µm were precipitated using droplet-based reactors, whilst sizes between 3.3 and 11.5 µm are achieved in continuous flow. This broadening effect has been noted previously for calcium carbonate precipitation in continuous flow by Ji et al.[112,113] In addition, the nucleation of crystals is heterogeneous at the microchannel walls, naturally associated with a lower energy barrier, and homogenous in the centre of the channel. Thus, the occurrence of homogeneous as well as heterogeneous nucleation inevitably results in different nucleation rates and subsequently a wide range in crystal size and polymorph.[139]

Under bulk synthesis conditions, localized variations in concentration across the reaction mixture leads to nucleation occurring over a greater time period. Since both, nucleation of new particles and growth of the already formed ones use free ions in the solution, these two processes are often in competition. Hence, particles with different sizes are often precipitated concurrently. With an increase in reagent concentration, the number of nuclei forming at a particular time increases. This reduces the number of free ions available to contribute to the growth of the previously-formed particles. This effect is evident in the precipitates formed when reagent concentrations are 8 mM and higher, where the large number of sub-micron precursor particles retards the growth of previously formed calcium carbonate, resulting in a smaller population of fully formed particles at any particular time. These findings are also in agreement with a small angle X-ray study of nucleation and growth of CaCO₃ performed by Bolze et al.,[140,141] where an increase in number density and in the rate of formation of small primary particles (ACC) was observed at higher reactant concentrations. Moreover, the presence of impurities and the container walls can promote heterogeneous nucleation, naturally associated with a lower energy barrier.[142] Thus, the occurrence of homogeneous as well as heterogeneous nucleation inevitably results in different nucleation rates and a wide
range of particle sizes and polymorph.

3.5 Conclusions

The use of a segmented flow microfluidic reactor for the controlled production of calcium carbonate crystal polymorphs has been demonstrated for the first time. Through the variation of reagent concentrations and the minimization of residence time distributions, high quality crystallites could be synthesized on short timescales. The most remarkable results were achieved for 4 and 8 mM reagent concentrations. Using reagent concentrations of 4 mM precipitation of only the calcite polymorph was observed. However, operation at concentrations of 8 mM yielded the complete stabilization of the vaterite polymorph at ambient conditions, and without the need for additives. In contrast, bulk and continuous flow microfluidic precipitation of calcium carbonate at the same concentrations and similar residence times yielded mixtures of both calcite and vaterite polymorphs.

Precipitation in droplets enables precise control of both nucleation and growth conditions producing particles of same the polymorph. In continuous flow formats, the parabolic velocity profile creates a distribution of residence times along the channel, thus producing crystals of different polymorph. In addition, bulk synthesis is usually associated with unequal mixing of reagents, which creates varying nucleation conditions for individual crystals, and thus results in a mixture of polymorphs. Accordingly it can be concluded that the segmented flow microfluidic platform used for the precipitation of calcium carbonates has clear advantages over other synthetic methods.
Chapter 4

Precipitation of Amorphous Calcium Carbonate

A droplet-based microfluidic system is used to investigate the early stages of CaCO$_3$ formation. Amorphous calcium carbonate is precipitated and isolated after growth periods of 2, 30, 60 and 120 s. The product is analysed by SEM to elucidate resulting size distributions and TEM to identify the amorphous phase. Control experiments are carried out in bulk solution with reaction volumes $10^6$ times larger than in droplets. It is found that amorphous calcium carbonate particles precipitated in droplets have a narrower size distribution than crystals precipitated from bulk solution. It is suggested that the transformation of the amorphous phase to the crystalline phase occurs via a dissolution-reprecipitation mechanism based on the reduction in size of the ACC after 120s.
4.1 Introduction

It is believed that under conditions of high supersaturation, the first step in the crystallisation pathway of calcium carbonate is the formation of amorphous calcium carbonate (ACC).[54] Generally, this unstable hydrated phase rapidly transforms to one of the anhydrous crystalline forms of CaCO$_3$, i.e. calcite, vaterite or aragonite.[54] Amorphous calcium carbonate is isotropic and can potentially be moulded into any shape prior to the crystallisation step, providing an attractive technique for the synthesis of new materials.[65,143]

Many biominerals such as those present in sea urchins and mollusc nacres are formed via an amorphous precursor and have remarkable properties (Figure 4.1).[16,63,144,145] For instance, formation of a sea urchin larval spicules occurs from the initial nucleation of a single crystal of calcite followed by deposition of a transient ACC phase that crystallises into a single (larger) crystal of calcite.[146] Similarly, during the formation of larvae of molluscan bivalves, the transient ACC precursor phase is transformed into aragonite.[147] The transformation of ACC to more thermodynamically stable polymorphs is often controlled by acidic proteins, and in some cases the amorphous form is permanently stabilised.[148]

![SEM images of various skeletal parts composed of stable ACC](image)

Figure 4.1 SEM images of various skeletal parts composed of stable ACC: (a) Body spicules from *Pyura pachydermatina*. (b) Cross section of a broken tunic spicule from *Pyura pachydermatina*. (c) Cystolith from the leaves of *Ficus microcarpa*. (d) Granule storage structure of *Orchestia cavimana*. Image taken from reference [16].
Due to its high solubility, thermodynamic instability and difficulty to detect, amorphous calcium carbonate has been overlooked in crystal growth studies, when it is associated with a crystalline phase.[16] It is particularly difficult to detect transient ACC that transforms with time into a crystalline polymorph. A common approach for studying amorphous calcium carbonate and crystallization pathways, without the use of additives, is the template directed use of Langmuir monolayers.[149,150] The presence of a monolayer lowers the energy barrier for nucleation and provides a heterogeneous nucleation pathway with enhanced control over nucleation and growth of calcium carbonate. Pichon et al. [151] used Cryo TEM as a quasi-time-resolved technique to study the early stages of calcium carbonate nucleation using a Langmuir monolayer. The authors found that the formation of ACC was followed by its growth, producing a population of larger particles that subsequently crystallized into vaterite (via solid state transformation) and then into calcite (via a dissolution-reprecipitation pathway). Lee et al. [152] studied the initial stages of mineral formation as well as the mechanism of the ACC to calcite transformation on a mercaptophenol self-assembled monolayer (SAM). They found that SAMs became covered with a layer of ACC particles that eventually transformed into predominantly calcite with some vaterite (<20%) via a dissolution-reprecipitation pathway. They observed dissolution of ACC as soon as the calcite crystal appeared on the ACC layer, with the solute ions from the dissolving ACC being transferred to the growing crystal.

Of particular relevance to the work described herein is the heterogeneous nucleation and growth of CaCO$_3$ within arrays of picoliter droplets formed on patterned SAMs.[64] Droplet arrays (Figure 4.2) were used to study crystal growth in a well defined and impurity free environment, where crystal growth could be spatially and chemically controlled. The authors were able to study growth and transformation of ACC into calcite without the use of any specialised analytical techniques. However, the specific orientation of the formed calcite crystals suggests that the transformation of ACC into calcite occurs on the SAM rather than in bulk solution.
Figure 4.2 Schematic describing the fabrication of droplet arrays. (a) A $1H,1H,2H,2H$-perfluorodecanethiol SAM deposited on a gold-on-mica substrate and exposed to UV light. (b) Removal of irradiated alkyl thiols with solvent. (c) “Backfilling” of exposed regions with mercaptohexadecanoic acid solution to yield a patterned surface. (d) Deposition of droplets by passing a Na$_2$CO$_3$/CaCl$_2$ solution across the SAM at 100% humidity. (e) Optical image of an array of 10 µm radius droplets. (f) Droplet with a radius $a$, height $h$, base radius $r$, and contact angle $\theta$ on the substrate. Image taken from reference [16].

The work described in this thesis considers homogeneous nucleation and subsequent growth of calcium carbonate. Accordingly another study relevant to the research presented herein is the precipitation of calcium carbonate in levitated droplets,[139] In this work the authors show that CaCO$_3$ precipitates homogeneously in a contact-free environment by vitrifying the droplet in liquid ethane, cryo-fracturing and analysing by Cryo SEM and TEM. The particles
collected from within the droplet and those formed at the droplet surface (liquid/air interface) have the same diameter. The homogeneous formation of CaCO$_3$ proceeded via an amorphous liquid-like state in the absence of any stabilising additives. These amorphous particles then serve as templates for the crystallisation of calcite.

To obtain ACC, the nucleus must be stabilised before it has a chance to transform into a more stable crystalline polymorph. This often means keeping its size below the critical size needed for transformation or preventing dehydration, as ACC must lose water for crystallisation to occur.[153] Interestingly Nudelman et al. [154] used a single flow microfluidic device to synthesise ACC at 4 °C and poly[(α,β)-DL-aspartic acid] to stabilise the formed ACC. They found that after 48 hours, particles that were smaller than 100 nm remained amorphous but those larger started to develop crystallinity.

Different methods have been reported for the generation of amorphous calcium carbonate; however, none of them has been successful in producing ACC that is stable whilst still in contact with an aqueous phase. Examples include the low temperature diffusion of calcium and carbonate ions,[65] the hydrolysis of dimethyl carbonate in a CaCl$_2$ solution,[155] and the fast mixing of saturated solutions of calcium salts and carbonates.[156] For instance, Rodriguez-Blanco et al.[137] hindered the transformation of ACC into crystalline CaCO$_3$ by performing the reaction at low temperatures (1°C). They obtained pure ACC consisting of spherical particles 50 to 200 nm in size, which were stable for 3 days. The effective stabilisation of the amorphous phase can be achieved by the use of additives, which control the kinetics of the mineralisation reaction. The mechanism of this stabilisation is thoroughly discussed in Chapter 5. For instance, the use of polyaspartate,[157] polyacrylate[158] and DNA[159] in this respect is well documented. Such polymeric additives have been observed to form thin films of calcium carbonate, which then transform into one of the crystalline polymorphs depending on the type of the additive used.[160]

In this work, the controlled precipitation and subsequent isolation of amorphous calcium carbonate was for the first time demonstrated in a segmented flow microfluidic system. The ability to arrest the reaction at early and precisely controlled times offers a method of studying nucleation and growth processes that proceed rapidly and uncontrollably in bulk solutions. Moreover, isolated amorphous calcium carbonate can potentially be further
moulded for the synthesis of materials with specific shape, polymorph and/or crystallographic orientation. The research presented herein is also of relevance to biomineralization processes, where crystallisation usually occurs in well-defined microenvironments.[64]

4.2 Experimental

4.2.1 Segmented Flow Microfluidic Synthesis

Small volumes of 8 mM solutions of CaCl₂ and Na₂CO₃ were delivered through the two inlets of a microfluidic device (Figure 4.3). The aqueous stream created by the two reagents was flow-focused with Mineral oil (Sigma-Aldrich) containing 2.5 % ABIL EM90 surfactant (Surfachem). Product was collected in a glass vial containing 10 ml of ethanol (Sigma Aldrich) to quench the reaction. Reaction times were altered by varying the length of PTFE tubing and the flow rates, such that the ratio of aqueous to oil flow rates remained constant. This ensured that the droplet volume always remains at 200 pL. Product was collected after 2, 30, 60 and 120 s. (Table 4.1) For a reaction time of 2 s, PTFE tubing was not required and the reaction mixture was collected directly from the outlet of the microfluidic chip into a vial containing ethanol. Hexane (Sigma Aldrich) was then added to the reaction mixture in order to dissolve the mineral oil. 1 ml of this solution was then deposited onto a 100 x 100 Cu grid (Agar Scientific), washed with ethanol and dried at room temperature. The rest of the solution was then filtered through a track-etch membrane (Millipore, 0.2 µm pore size), washed with ethanol and dried at room temperature overnight.
Figure 4.3  Schematic of the microfluidic system used for droplet-based microfluidic synthesis of amorphous calcium carbonate. Droplets are formed by flow focusing two aqueous streams of reagents using Mineral oil. The droplets then enter PTFE tubing and are quenched by a large volume of ethanol.

<table>
<thead>
<tr>
<th>Residence time (s)</th>
<th>Length of the PTFE tubing (cm)</th>
<th>Aqueous flow rate (µl/min)</th>
<th>Mineral oil flow rate (µl/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>0</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>30</td>
<td>40</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>60</td>
<td>40</td>
<td>0.5</td>
<td>1</td>
</tr>
<tr>
<td>120</td>
<td>40</td>
<td>0.25</td>
<td>0.5</td>
</tr>
</tbody>
</table>

Table 4.1: Experimental parameters for experiments at each residence time.

4.2.2 Bulk Synthesis

100 ml of 8 mM solutions of CaCl$_2$ and Na$_2$CO$_3$ were mixed together and shaken vigorously for 30, 60 and 120 s in separate experiments. Part of the reaction mixture was then filtered through a track-etch membrane, washed with ethanol and dried at room temperature. 1 ml of the solution was deposited onto a 100 x 100 Cu grid, washed with ethanol and dried at room temperature.
4.2.3 Product Analysis

Particles on track-etch membranes were sputter-coated with chromium for SEM imaging. Particle size distributions were determined from SEM images using ImageJ software, following the procedure defined in Chapter 2. Particle size distribution, mean size and population standard deviation were obtained by analysing at least 100 particles. Particles on a copper grid were imaged using TEM for high magnification morphological analysis with particle crystallinity determined using electron diffraction. All experiments were repeated at least three times.

4.3 Results

4.3.1 Segmented Flow Microfluidic Synthesis

Calcium carbonate was precipitated by combining equal volumes of 8 mM CaCl$_2$ and 8 mM Na$_2$CO$_3$ in microfluidic droplets. This concentration was chosen because at lower concentrations the reaction rate is too slow. To increase residence times, either lower flow rates or longer lengths of PTFE tubing are required, neither of which are conducive to stable droplet formation and flow. At flow rates below 0.25 µl/min, droplet flow becomes unstable resulting in large variations in droplet volume and large gaps between the droplets due to temporary disruptions in the flow. Additionally, when using longer lengths of PTFE tubing, the pressure increase in the system leads to occasional back flow of the solutions, creating unstable droplet flow. When using reagent concentrations above 8 mM, particles precipitate at the beginning of the channel where the two streams mix. This leads to blockage of the device. When using reagent concentrations of 8 mM or lower, no precipitation on the channel walls was observed.

Particles collected 2 s after reaction commencement were spherical in shape (Figure 4.4) and between 43.9 and 82.9 nm in size with a mean particle diameter of 64.1 nm and a population
The standard deviation of 4.3 nm (Figure 4.4 E and Table 4.2). The obtained diffraction pattern was characteristic of an amorphous material showing diffuse rings (Figure 4.4 C). Upon longer exposure by the electron beam, the appearance of polycrystalline rings on the diffraction pattern indicated that crystallisation of the amorphous calcium carbonate into a more ordered structure has occurred (Figure 4.4 D). \(d\)-spacings were assigned to the (112) and (300) crystal planes of vaterite.

![Figure 4.4](image)

**Figure 4.4** (A) SEM and (B) TEM micrographs of CaCO\(_3\) particles precipitated in droplets from 8 mM Na\(_2\)CO\(_3\) and CaCl\(_2\) after a 2 s growth period. (C) SAED pattern for CaCO\(_3\) precipitate on a TEM grid showing the broad rings characteristic of amorphous calcium carbonate. (D) SAED pattern for the same CaCO\(_3\) precipitate showing the polycrystalline domains formed upon longer exposure of the particles by the electron beam. (E) Size distribution of the precipitated amorphous CaCO\(_3\).
Particles collected after a growth time of 30 s were also spherical with a diameter ranging from 77.5 to 125.5 nm (Figure 4.5) The mean diameter was found to be 101.1 nm, with a population standard deviation of 4.7 nm (Figure 4.5 and Table 4.2). The initial diffraction pattern consisted of broad rings, characteristic of an amorphous material, which turned into more defined polycrystalline rings upon longer exposure of the sample to the electron beam (Figure 4.5 C, D). d-spacings were assigned to the (114), (304) and (401) crystal planes of vaterite.

Figure 4.5  (A) SEM and (B) TEM micrographs of CaCO$_3$ particles precipitated in droplets from 8 mM Na$_2$CO$_3$ and CaCl$_2$ after a 30 s growth period. (C) SAED pattern for CaCO$_3$ precipitate on a TEM grid showing the broad rings characteristic of amorphous calcium carbonate. (D) SAED pattern for the same CaCO$_3$ precipitate showing the polycrystalline domains formed upon exposure of the particles to the electron beam. (E) Size distribution of the precipitated amorphous CaCO$_3$. 

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Particles collected after a growth time of 60 s had a particle size distribution ranging from 76.8 to 154.4 nm, a mean diameter of 114.3 nm and a population standard deviation of 7.5 nm (Figure 4.6, Table 4.2). The diffraction pattern was initially characteristic of an amorphous phase, and upon longer exposure to the electron beam characteristic of a polycrystalline phase (Figure 4.6 C,D) showing the (114), (224) and (412) planes of vaterite.

Figure 4.6  (A) SEM and (B) TEM micrographs of CaCO$_3$ particles precipitated in droplets from 8 mM Na$_2$CO$_3$ and CaCl$_2$ after a 60 s growth period. (C) SAED pattern for CaCO$_3$ precipitate on a TEM grid showing the broad rings characteristic of amorphous calcium carbonate. (D) SAED pattern for the same CaCO$_3$ precipitate showing the polycrystalline domains formed upon exposure of the particles to the beam. (E) Size distribution of the precipitated amorphous CaCO$_3$. 
Particles collected after a growth time of 120 s had a mean diameter of 83.9 nm ranging from 61.0 to 107.2 nm and a population standard deviation of 4.8 nm (Figure 4.7, Table 4.2). Again, the initial diffraction pattern consisted of broad rings characteristic of an amorphous phase. Upon longer exposure, the amorphous phase began to crystallise (Figure 4.7 C, D), with \(d\)-spacings assigned to the (114) (211), (224) and (228) crystal planes of vaterite.

![Image](image-url)

Figure 4.7 (A) SEM and (B) TEM micrographs of CaCO\(_3\) particles precipitated in droplets from 8 mM Na\(_2\)CO\(_3\) and CaCl\(_2\) after a 120 s growth period. (C) SAED pattern for CaCO\(_3\) precipitate on a TEM grid showing the broad rings characteristic of amorphous calcium carbonate. (D) SAED pattern for the same CaCO\(_3\) precipitate showing the polycrystalline domains formed upon exposure of the particles to the beam. (E) Size distribution of the precipitated amorphous CaCO\(_3\).
<table>
<thead>
<tr>
<th>Residence time (s)</th>
<th>Size range (nm)</th>
<th>Mean size (nm)</th>
<th>Standard Deviation (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>43.9 - 82.9</td>
<td>64.1</td>
<td>4.3</td>
</tr>
<tr>
<td>30</td>
<td>77.5 - 127.5</td>
<td>101.1</td>
<td>4.7</td>
</tr>
<tr>
<td>60</td>
<td>76.8 - 154.4</td>
<td>114.3</td>
<td>7.5</td>
</tr>
<tr>
<td>120</td>
<td>61.0 - 107.2</td>
<td>83.9</td>
<td>4.8</td>
</tr>
</tbody>
</table>

Table 4.2: Size distribution, mean size and standard deviation for amorphous calcium carbonate collected after 2, 30, 60 and 120 s growth periods within droplets.

### 4.3.2 Bulk Synthesis of ACC

CaCO$_3$ synthesis was also performed on the macroscale with reaction volumes 10$^6$ times larger than the droplets formed in the microfluidic device. Particles were made by mixing 100 ml of 8 mM CaCl$_2$ and 100 ml of 8 mM Na$_2$CO$_3$ in a beaker with vigorous shaking for the same (residence) times employed in the microfluidic syntheses. It should be noted that it was only possible to collect particles after a 30 s due to the difficulties associated with isolating product after 2 s.

Particles collected after 30 s were spherical in shape (Figure 4.8), with diameters ranging from 50.9 to 129.6 nm, a mean diameter of 87.4 nm and a population standard deviation of 7.6 nm (Figure 4.8, Table 4.3). The diffraction pattern was characteristic of an amorphous material (Figure 4.8). Upon exposure to the electron beam, the appearance of polycrystalline rings on the diffraction pattern indicated crystallisation of the amorphous phase with observation of the (004), (114) and (308) planes of vaterite (Figure 4.8 D).
Figure 4.8  (A) SEM and (B) TEM micrographs of CaCO$_3$ particles precipitated in bulk solution from 8 mM Na$_2$CO$_3$ and CaCl$_2$ after a 30 s growth period. (C) SAED patterns for CaCO$_3$ precipitate on a TEM grid showing the broad rings characteristic of amorphous calcium carbonate. (D) SAED pattern for the same CaCO$_3$ precipitate showing the polycrystalline domains formed upon exposure of the particles to the electron beam. (E) Size distribution of the precipitated amorphous CaCO$_3$.

After a 60 s reaction time, amorphous particles having diameters ranging from 64.4 and 150.9 nm, a mean diameter of 103.5 nm and a population standard deviation of 9.22 nm (Figure 4.9, Table 4.3) were formed. Particles were observed to crystallise upon exposure to the electron
beam, indicated by the presence of polycrystalline rings on the diffraction pattern (Figure 4.9 C, D). \(d\)-spacings were assigned to the (112), (114), (222) and (0012) crystal planes of vaterite.

Figure 4.9 (A) SEM and (B) TEM micrographs of CaCO\(_3\) particles precipitated in bulk solution from 8 mM Na\(_2\)CO\(_3\) and CaCl\(_2\) after a 60 s growth period. (C) SAED patterns for CaCO\(_3\) precipitate on a TEM grid showing the broad rings characteristic of amorphous calcium carbonate. (D) SAED pattern for the same CaCO\(_3\) precipitate showing the polycrystalline domains formed upon exposure of the particles to the beam. (E) Size distribution of the precipitated amorphous CaCO\(_3\).
After a reaction time of 120 s, the size of the formed amorphous particles decreased slightly, with particles having sizes between 66.8 to 137.4 nm, a mean diameter of 104.0 nm and a population standard deviation of 6.8 nm (Figure 4.10, Table 4.3). The broad rings on the diffraction pattern indicated the presence of an amorphous phase, which began to crystallise upon exposure to the electron beam (Figure 4.10 C,D). \( d \)-spacings were assigned to the (112), (304), (224) and (410) crystal planes of vaterite.

![Figure 4.10](image)

(A) SEM and (B) TEM micrographs of CaCO\(_3\) particles precipitated in bulk solution from 8 mM Na\(_2\)CO\(_3\) and CaCl\(_2\) after an 120 s growth period. (C) SAED patterns for CaCO\(_3\) precipitate on a TEM grid showing the broad rings characteristic of amorphous calcium carbonate. (D) SAED pattern for the same CaCO\(_3\) precipitate showing the polycrystalline domains formed upon exposure of the particles to the electron beam. (E) Size distribution of the precipitated amorphous CaCO\(_3\).
<table>
<thead>
<tr>
<th>Residence time (s)</th>
<th>Size range (nm)</th>
<th>Mean size (nm)</th>
<th>Standard Deviation (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>30</td>
<td>50.9 – 129.6</td>
<td>87.4</td>
<td>7.6</td>
</tr>
<tr>
<td>60</td>
<td>64.4-150.9</td>
<td>103.5</td>
<td>9.2</td>
</tr>
<tr>
<td>120</td>
<td>66.8 - 137.4</td>
<td>104.0</td>
<td>6.8</td>
</tr>
</tbody>
</table>

Table 4.3: Size distribution, mean size and standard deviation for amorphous calcium carbonate particles collected after 30, 60 and 120 s reaction times and precipitated from bulk solution.

4.4 Discussion

As discussed in Chapter 3, in well-defined droplets, nucleation occurs over a short period of time since ions have to travel short distances before reacting. If nucleation is short, then all nuclei will form at the same time and will have the same amount of time to grow. Growth is usually limited by diffusion of solutes to the nucleus, and since droplets provide for uniform solute concentrations the growth rate in each droplet is likely to be similar. This in turn should result in precipitated particle populations having small size distributions. For instance, after 30 s of growth, the size of the particles precipitated in droplets ranged from 77.5 to 127.5 nm, whereas particles precipitated in bulk ranged between 50.9 to 129.6 nm. The population standard deviation for precipitation in droplets was 4.7 nm compared to 7.6 nm in bulk solution. After a 60 s growth period, particles precipitated in droplets were sized between 76.8 and 154.4 nm, whereas particles precipitated in bulk ranged from 64.4 to 150.9 nm, with the population standard deviations being 7.5 nm and 9.2 nm respectively. After 120 s, particles precipitated in droplets had sizes between 61.0 and 107.2 nm and those in bulk between 66.8 and 137.4 nm. The population standard deviation for precipitation in droplets was 4.8 nm compared to 6.3 nm for precipitation in bulk solution. Furthermore, it was possible to isolate ACC at very early times in droplets (2 s). This proved impossible to achieve in bulk solution due to the challenges associated with handling solutions and quenching reactions on short timescales.
The data also demonstrate faster rates of reaction in pL-volume droplets when compared to reaction in bulk solution. For example, after a 30 s growth period, the mean size for particles precipitated in droplets was 101.1 nm compared to 87.4 nm for those precipitated in bulk solution. After a 60 s growth time, particles precipitated in droplets had an average size of 114.3 nm, whereas particles precipitated in bulk solution had an average size of 103.5 nm. When compared to bulk systems, the diffusive mixing in droplets is faster.[18] After a 120 s growth period, however, the average particle diameter precipitated by both methods decreased. The mean particle diameter for the droplet synthesis was 83.9 nm and 104.0 nm for the bulk synthesis. The decrease in particle size for reaction times of 120 s both in droplet and bulk syntheses can be attributed to the dissolution of ACC prior to calcite/vaterite formation upon reaching a critical nuclei size. Diffraction patterns taken from various areas on the grid after 120 s for both bulk and droplet-based synthesis were more crystalline and less amorphous, confirming the beginning of the formation of a more thermodynamically stable polymorph. The high solubility of ACC is the driving force for dissolution and it is likely that calcite/vaterite formation occurs via a dissolution-reprecipitation mechanism,[140,161,162] where the solute ions from dissolving ACC are transferred to the growing crystal. Accordingly a more stable polymorph of the calcium carbonate grows at the expense of ions released from the dissolution of the amorphous phase. The evidence that calcium carbonate sometimes precipitates via a dissolution-reprecipitation pathway had been demonstrated previously. For example, Bolze et al. used time-resolved small-angle and wide-angle X-ray scattering experiments (TR-SAXS/WAXS) to demonstrate that the transformation of the ACC proceeds via dissolution and subsequent heterogeneous nucleation on the walls of a quartz capillary.[140,162] Likewise, Lee et al. used organothiol self-assembled monolayers (SAMs) to study the early stages of CaCO₃ formation and confirmed that the transformation from amorphous to crystalline phase occurs via a dissolution-reprecipitation pathway.[152]

The mean size of the ACC precipitated in droplets is significantly lower after 120 s than that of the ACC precipitated from bulk (83.9 nm versus 104.0 nm). When the ACC has grown to its critical size at the expense of the substituent ions, the concentration of the Ca²⁺ and CO₃²⁻ ions in droplets will be low. This causes ACC to dissolve and lose substituent ions relatively quickly due to the low concentration of the ions in droplets. In bulk solution, the initial
formation of ACC from its ions occurs on a longer timescale. Thus, when ACC has reached its critical size, there are still ions present in a solution and the dissolution of ACC occurs at a slower rate.

A plot of the average size of ACC precipitated at different times in both droplet and bulk is shown in Figure 4.11.

![Graph of average particle size versus reaction time for droplet-based synthesis and bulk synthesis of amorphous calcium carbonate.](image)

The data presented herein suggest that the crystallisation of CaCO₃ both in droplets and bulk solutions proceeds in two steps. Firstly, amorphous particles are formed from ions by homogeneous nucleation. Upon reaching a critical size, they slowly dissolve again releasing the substituent ions. These ions then form a more stable polymorph of CaCO₃.

Unfortunately, it was not possible to study the growth and dissolution of ACC further due to disturbances in the droplet flow associated with higher residence times. When using longer PTFE tubing to increase residence times, the pressure increase in the system leads to
occasional back flow of the solutions, creating unstable droplet flow. This changes the experimental conditions as the droplet volume becomes variable.

4.5 Conclusion

In this chapter, the versatility and applicability of droplet-based microfluidic synthesis has been further demonstrated by its application to the synthesis of amorphous calcium carbonate. These experiments represent the first reported use of droplet-based microfluidic devices for the synthesis and isolation of amorphous calcium carbonate without the use of additives. The ability to arrest a chemical reaction in a microfluidic device at very early stages provides a unique platform for the step-by-step study of nucleation and growth of particles. The ability to tune residence times with a precision of a few seconds eliminates human error from the reaction procedure. In addition, the fact that nucleation occurs on shorter timescales in droplets means that narrower size distributions of the synthesised particles are obtained when compared to bulk systems. Finally, the presence of localised ion concentration gradients in droplets enables the dissolution of ACC to occur at a faster rate than in bulk solution.
Studies reported in this chapter discuss the process of polymer-mediated calcium carbonate formation. Specifically the concentration of poly (4-sodium styrene sulfonate) was varied in order to assess its effect on the crystallisation process. Particles were analysed using TEM and SEM. This dual analysis approach allows observation of the early stages of crystallisation, and characterisation of the final product.
5.1 Introduction

The ability to achieve controlled and reproducible crystallisation is an immense challenge since crystal size, shape, texture and polymorph define application in a wide range of industries. Accordingly, crystal nucleation and growth have been widely investigated. In addition to the classical model of crystallisation, where growth is defined by an amplification process in which a single nucleus is enlarged without any structural change in the bulk or at the surface,[50] there are also non-classical pathways that progress via colloidal intermediates and mesoscale transformation.[50,163,164] Mesoscale assembly occurs by aggregation of single, pre-formed crystalline nanoparticles resulting in a single crystal with an iso-orientated mosaic texture (Figure 5.1).[50] The subsequent internal rearrangement of these nanoparticles produces a crystallographically continuous particle. The crystalline subunits are often arranged in perfect three-dimensional order, making it difficult to distinguish between a mesocrystal and a single crystal, since both show identical scattering patterns and behaviour in polarized light.[165] However, microscopic images do show subunits with mesoscopic size when a mesocrystal is present.

![Diagram](image)

Figure 5.1 Two mechanisms for growth of a single crystal: classic crystallisation and mesoscale assembly. Image taken from reference [50].
Colfen et al. have discussed the possible influence of polymers on the process of crystallization (Figure 5.2).[166] They propose that polymers can block or slow down crystal growth by complex formation, thus making assembly effects (Figure 5.2 b,c) more important than classical crystallisation (Figure 5.2 a). Polymers can also cause an increase in the number of primary nanoparticles by acting as a nucleation agent (Figure 5.2 e), stabilizing an amorphous precursor phase (Figure 5.2 c) and causing a change in the shape of primary nanoparticles by selective adsorption and/or enrichment onto specific crystal faces (Figure 5.2 d). Usually, however, the polymer adopts more than one role, making it very difficult to predict the final crystal morphology.[166]

![Figure 5.2](image.png)

**Figure 5.2** Crystallisation pathways by: addition of ions (a), mesoscale assembly (b), stabilising amorphous precursor phase (c), acting as a nucleation agent (e) and selective adsorption (d). Image taken from reference [166].

While the mechanism of mesocrystal formation and the forces that control it are unknown,[167] several mesocrystal formations have been discovered. One of the first examples of mesoscale assembly was observed in 1969 by Petres et al. during the formation of a porous internal structure of BaSO₄.[168] However, according to classical crystallization theory, the compound should crystallise to form a defect-free single crystal.[1] In 1988, Matijevic et al. synthesised various Ce⁴⁺ compounds, without any additives, and found the structure of the
product to consist of nanoparticle aggregates.[169] More recently, Colfen et al. used a block copolymer consisting of ionic and non-ionic segments to enable selection between the polycrystalline, mesocrystalline and single crystalline states of calcium carbonate by varying the concentrations of both copolymer and calcium.[114] There is also evidence that some biominerals are indeed mesocrystals. In a recent study, atomic force microscopy (AFM) analysis of the fracture pattern of sea urchin skeletal elements found a rough, cluster like pattern that scattered like a single crystal, but had a nearly isotropic fracture behaviour, which is not typical for single-crystalline calcite.[170]

Many of the most defined mesocrystals are observed in gels.[167] Gels minimize convection and turbulence during the crystallisation process, thus enhancing the interaction potential between primary particles and reducing the mutual alignment governed by the change in environment. Hydrogels for example isolate solutes in small pores and growth does not start until the supersaturation of ions is relatively high.[167] High supersaturation leads to an increase in nucleation of primary particles, the building blocks of mesocrystals.[171] Of relevance to the work herein is the growth of CaCO$_3$ in polyacrylamide gels.[172,173] In these studies, calcite crystals were formed having characteristic pseudo-octahedral morphologies. The alignment of individual crystals was shown by TEM (Figure 5.3 B). A growth model was proposed where hierarchical aggregation of rhombohedral subunits forms the calcite mesocrystal.

![Figure 5.3](image_url)  
(A) SEM micrograph of a calcite aggregate grown in polyacrylamide gel. (B) TEM image of a microstructure showing alignment of individual crystallites (inset: electron diffraction pattern of a calcite crystal). Image taken from reference [172].
In a similar way confinement provided by droplet-based microfluidic systems can potentially be useful in studying nucleation pathways for different materials. In the current work calcium carbonate was precipitated in the presence of (poly (4-sodium styrene sulfonate) (PSS) in a droplet-based microfluidic device. Crystals were analysed at early growth times and in a thermodynamically stable state. Droplet-based microfluidic reactors are ideal for studying the early stages of the crystallization reaction of CaCO₃ since they provide an even better reaction environment than gels. For example, in droplets, even higher supersaturation can be achieved through the use of extremely small droplet volumes and high reagent concentrations. Droplets also provide a homogeneous reaction environment, thus ensuring minimal interactions between primary particles and surfaces. In contrast, precipitation in gels necessarily involves the addition of an extra chemical component, which inevitably influences the growth of crystals. Finally, the use of droplets offers a degree of control over reaction times that is not possible in conventional systems.

5.2 Experimental

5.2.1 Segmented Flow Microfluidic Synthesis

A stock solution of CaCl₂ (10 mM) was freshly prepared in a conical flask. From this stock solution, 10 ml solutions were mixed with 10, 50 and 100 µl/mg concentrations of poly(4-sodium styrene sulfonate) (MW ≈ 70 000 g/mol, Sigma-Aldrich).

Volumes of the 10 mM Ca-PSS complex and 10 mM Na₂CO₃ were then delivered through the two inlets of a microfluidic device (as described in Figure 4.3). Mineral oil (Sigma-Aldrich) containing 2.5 % ABIL EM90 surfactant (Surfacem) was used to flow-focus the aqueous stream. Product was collected in a glass vial containing ethanol (10 ml, Sigma Aldrich) to quench the reaction. Residence times were adjusted between 2 and 120 s by varying the length of PTFE tubing and the flow rates of the input streams (see Table 4.1). The volume of the droplets was kept constant at 200 pL. Hexane (Sigma Aldrich) was then added
to the reaction mixture to dissolve the mineral oil. The resulting solution was subsequently deposited onto 100 x 100 Cu grids (Agar Scientific), washed with ethanol and dried at room temperature overnight.

To obtain the final precipitation product at each concentration of PSS, the reaction mixture was allowed to drip from the PTFE tubing into an empty glass vial and left to crystallise for 1 day. Hexane was then added to each vial to dissolve the mineral oil. The resulting solution was then filtered through a track-etch membrane (Millipore, 0.2 µm pore size), washed with ethanol and dried at room temperature overnight.

### 5.2.3 Analysis

The early stages of crystallisation were analysed by TEM in the same manner as described in Section 4.2.3. The final product was analysed by SEM as described in Section 3.2.4.

### 5.3 Results

Initially, the effect of poly(4-sodium styrene sulfonate) on the precipitation of calcium carbonate was studied at early reaction times, before allowing the product to crystallise into its thermodynamically stable state. 10 mM reagent concentrations were chosen because, when using lower concentrations, the reaction proceeds at a slower rate requiring longer residence times. When using a higher reagent concentration, particles slowly precipitated at the beginning of the channel, where the two input streams meet, resulting in shorter operational lifetimes of the microfluidic devices. The effect of the polymer, mixed with CaCl₂, was studied at polymer concentrations of 10, 50 and 100 µg/ml.

Particles formed in the presence of 10 µg/ml PSS and collected after 2 s were less than 50 nm in diameter and spherical in shape (Figure 5.4 A). The diffraction pattern taken from the area indicated by a white circle (Figure 5.4 B) shows diffuse rings, indicative of the amorphous nature of the product.
Product collected after 30 s and precipitated in the presence of 10 µg/ml PSS, consisted of small spherical nanoparticles, with diameters below 50 nm, (indicated by ‘1’ in Figure 5.5 A), larger spherical nanoparticles (greater than 50 nm in diameter) and an area of accumulation of larger nanoparticles (indicated by ‘2’ in Figure 5.5 A). Electron diffraction patterns originating from the areas of smaller and larger nanoparticles consisted of broad rings characteristic of an amorphous material (ACC) (Figure 5.5 B). Diffraction patterns originating from the area of accumulated nanoparticles, however, consisted of broad amorphous rings overlaid with a spot pattern corresponding to the $d$-spacings of vaterite.
(Figure 5.5 C). Interatomic distances of 3.13, 2.56 and 1.64 Å were assigned to the (1 0 7), (1 1 7) and (3 1 5) lattice planes of vaterite respectively.

Figure 5.5  (A) Micrograph of CaCO₃ particles precipitated from 10 mM Na₂CO₃ and CaCl₂ with 10 µg/ml PSS after a 30 s growth period. (B) Corresponding SAED pattern of the domain labelled (1) on the TEM micrograph showing its amorphous nature. (C) Indexed SAED pattern of the domain labelled (2) on the TEM micrograph showing its amorphous nature (diffuse rings), with polycrystalline domains and calculated d-spacings corresponding to vaterite.

Product collected after 60 s and precipitated in the presence of 10 µg/ml PSS, consisted of small spherical nanoparticles (indicated by the white oval in Figure 5.6 C), large spherical particles (Figure 5.6 C) and shapeless “sheets” (Figure 5.6 A). Diffraction patterns originating from the “sheets” showed single crystal character with a spot pattern
corresponding to the $d$-spacings of calcite (Figure 5.6 B). Interatomic distances of 2.25, 2.19, 1.67, 1.47 and 1.33 Å were assigned to the (1 1 3), (2 0 2), (2 1 1), (1 2 5), (0 2 10) lattice planes of calcite respectively. The diffraction pattern originating from large spherical particles showed polycrystalline rings corresponding to the $d$-spacings of vaterite (Figure 5.6 D). Interatomic distances of 3.45, 2.67, 2.00 and 1.75 Å were assigned to the (1 1 2), (2 0 5), (3 0 3) and (2 2 4) lattice planes of vaterite respectively.

Figure 5.6  
(A) TEM micrograph of CaCO$_3$ particle precipitated from 10 mM Na$_2$CO$_3$ and CaCl$_2$ with 10 µg/ml PSS after a 60 s growth period. (B) Indexed single crystal SAED pattern with calculated $d$-spacings corresponding to calcite. (C) TEM micrograph of spherical particles. The white oval shows the presence of nanoparticles. (D) Indexed SAED pattern with calculated $d$-spacings corresponding to vaterite.
After a reaction time of 120 s, product precipitated in the presence of 10 µg/ml PSS consisted of small nanoparticles (indicated by a white oval in Figure 5.7 C), large spherical nanoparticles (Figure 5.7 C) and shapeless “sheets” of material (Figure 5.7 A). The inset of Figure 5.7 A also shows the lattice fringes that were observed under high-resolution TEM. The electron diffraction pattern taken from an area of the “sheet” material showed single crystal character with a spot pattern corresponding to the $d$-spacings of calcite (Figure 5.7 B). The interatomic distances of 3.13 and 2.27 Å were assigned to the (1 0 4) and (1 1 3) lattice planes of calcite respectively. Diffraction patterns from the large spherical particles consisted of polycrystalline rings corresponding to the $d$-spacings of vaterite (Figure 5.7 D). The interatomic distances of 2.86, 2.50, 1.64 and 1.39 Å were assigned to the (1 0 8), (2 0 6), (1 0 15) and (3 2 5) lattice planes of vaterite respectively.

Figure 5.7  
A) TEM micrograph of CaCO$_3$ particles precipitated from 10 mM Na$_2$CO$_3$ and CaCl$_2$ with 10 µg/ml PSS after a 120 s growth period, the inset shows interplanar distances. (B) Indexed single crystal SAED pattern with calculated $d$-spacings corresponding to calcite. (C) TEM micrograph of spherical particles, with the white oval showing the presence of nanoparticles. (D) Indexed polycrystalline SAED pattern with calculated $d$-spacings corresponding to vaterite.
Particles formed in the presence of 50 µg/ml PSS and collected after 2 s were less than 50 nm in diameter, spherical (Figure 5.8 A) and amorphous as indicated by the diffuse rings on a diffraction pattern originating from the area marked with a white oval in Figure 5.8 A.

![Figure 5.8](image)

**Figure 5.8**  (A) TEM micrograph of CaCO$_3$ particles precipitated from 10 mM Na$_2$CO$_3$ and CaCl$_2$ with 50µg/ml PSS after a 2 s growth period. (B) Corresponding SAED pattern of the domain indicated by a white oval on the TEM micrograph and showing its amorphous nature.

Product collected after 30 s and precipitated in the presence of 50 µg/ml PSS, consisted of small spherical nanoparticles which were less than 50 nm in diameter (indicated by ‘1’ in Figure 5.9 A), larger spherical nanoparticles and densely accumulated nanoparticles (indicated by ‘2’ in Figure 5.9 A). Electron diffraction patterns originating from areas populated by small and large nanoparticles consisted of broad rings characteristic of
amorphous calcium carbonate (Figure 5.9 B). The diffraction pattern originating from accumulated nanoparticles consisted of broad amorphous rings overlaid with spot patterns (Figure 5.9 C) corresponding to the $d$-spacings of vaterite. The interatomic distances of 2.67 and 2.04 Å were assigned to the (2 0 5) and (3 0 3) lattice planes of vaterite respectively.

![Image](image_url)

Figure 5.9  (A) TEM micrograph of CaCO$_3$ particles precipitated from 10 mM Na$_2$CO$_3$ and CaCl$_2$ with 50 µg/ml PSS after a 30 s growth period. (B) Corresponding SAED pattern of the domain labelled (1) on the TEM micrograph showing its amorphous nature. (C) Indexed SAED pattern of the domain labelled (2) on the TEM micrograph showing its amorphous nature (diffuse rings) with polycrystalline domains and calculated $d$-spacings corresponding to vaterite.

For a 60 s reaction time, calcium carbonate precipitated in the presence of 50 µg/ml PSS consisted of small nanoparticles indicated by a white circle in Figure 5.10 C, shapeless “sheets” of material (Figure 5.10 A) and large spherical particles. It was possible to see the lattice fringes that developed in a few places of the “sheet” material (Figure 5.10 A, inset). In
Figure 5.10 C, the transformation of vaterite into a more ordered crystal is captured. A similar transformation was reported by Pouget et al. [174] in a template-controlled formation study using Cryo-TEM. In this study the authors observed how polycrystalline spherical vaterite transformed into a more developed crystal with a single crystal diffraction pattern characteristic of vaterite. The electron diffraction pattern of the “sheet” material showed single crystal character with a $d$-spacing corresponding to calcite (Figure 5.10 B). The interatomic distances of 2.25, 2.63, 1.71 and 1.33 Å were assigned to the $(113)$, $(110)$, $(211)$ and $(0210)$ lattice planes of calcite respectively. The electron diffraction pattern for particles undergoing transformation showed a dominant diffraction pattern with $d$-spacings corresponding to vaterite (Figure 5.10 D). The interatomic distances of 3.38, 1.83 and 1.20 Å were assigned to the $(113)$, $(220)$ and $(333)$ lattice planes of vaterite respectively.

Figure 5.10

(A) TEM micrograph of CaCO$_3$ particles precipitated from 10 mM Na$_2$CO$_3$ and CaCl$_2$ with 50 µg/ml PSS after a 60 s growth period showing shapeless “sheets”. The inset shows interplanar distances of the area indicated by the white oval. (B) Indexed single crystal SAED pattern with calculated $d$-spacings corresponding to calcite. (C) TEM micrograph of microstructure showing the alignment of individual crystallites, the white oval shows the presence of nanoparticles. (D) Indexed SAED pattern with calculated $d$-spacings corresponding to vaterite.
After a reaction time of 120 s, the product precipitated in the presence of 50 µg/ml PSS consisted of small nanoparticles (indicated by a white oval in Figure 5.11 A), large spherical particles (Figure 5.11 A) and shapeless “sheets” of material (Figure 5.11 C). The electron diffraction pattern originating from the spherical particles consisted of polycrystalline rings corresponding to the \(d\)-spacings of vaterite (Figure 5.11 B). The interatomic distances of 2.98, 2.46, 2.04 and 1.72 Å were assigned to the \((1\,1\,5)\), \((2\,0\,6)\), \((3\,0\,3)\) and \((1\,1\,13)\) lattice planes of vaterite respectively. The electron diffraction pattern of the “sheet” material showed single crystal character with a \(d\)-spacings corresponding to calcite (Figure 5.11 D). The interatomic distances of 2.94, 1.46, 1.37 and 1.11 Å were assigned to the \((0\,0\,6)\), \((3\,0\,0)\), \((2\,1\,7)\) and \((1\,3\,4)\) lattice planes of calcite respectively.

![Figure 5.11](image)

Figure 5.11  (A) TEM micrograph of CaCO\(_3\) particles precipitated from 10 mM Na\(_2\)CO\(_3\) and CaCl\(_2\) with 50 µg/ml PSS after a 120 s growth period, the white oval shows the presence of nanoparticles. (B) Indexed SAED pattern with calculated \(d\)-spacings corresponding to vaterite. (C) TEM micrograph of another particle showing shapeless “sheets” and indexed single crystal SAED pattern (D) with calculated \(d\)-spacings corresponding to calcite.
Upon increasing the amount of PSS to 100 µg/ml, particles collected after 2 s displayed the same characteristics as when using lower concentrations of PSS (Figure 5.12 A). They were less than 50 nm in diameter, spherical and amorphous in nature, as shown by diffuse rings on the diffraction patterns originating from the area marked with a white oval in Figure 5.12 B.

![Figure 5.12](image)

(A) TEM micrograph of CaCO₃ particles precipitated from 10 mM Na₂CO₃ and CaCl₂ with 100 µg/ml PSS after a 2 s growth period. (B) Corresponding SAED pattern of the domain indicated on the TEM micrograph showing its amorphous nature.

Product collected after 30 s and precipitated in the presence of 100 µg/ml PSS, consisted of small spherical nanoparticles which were less than 50 nm in diameter (indicated by ‘1’ in Figure 5.13 A), large spherical nanoparticles (Figure 5.13 A) and an area of densely accumulated nanoparticles (indicated by ‘2’ in Figure 5.13 A). The electron diffraction pattern verified that the small and large nanoparticles were amorphous in nature, as judged by
the broad rings (Figure 5.13 B). The diffraction pattern originating from accumulated nanoparticles consisted of broad amorphous rings overlaid with a spot pattern (Figure 5.13 C) corresponding to the $d$-spacings of vaterite. The interatomic distances of 2.67, 1.55 and 1.43 Å were assigned to the (2 0 5), (4 0 3) and (3 2 2) lattice planes of vaterite respectively.

Product collected after 60 s in the presence of PSS consisted of a mixture of small nanoparticles (indicated by a white circle on Figure 5.14 A), large spherical particles (Figure 5.14 A) and shapeless “sheets” (Figure 5.14 C). The electron diffraction patterns originating from large spherical particles were indexed and the values of the $d$-spacings confirmed the
The presence of the vaterite polymorph (Figure 5.14 B). The interatomic distances of 3.33, 2.50 and 1.94 Å were assigned to the (1 1 3), (2 0 6) and (3 0 5) lattice planes of vaterite respectively. Electron diffraction patterns originating from shapeless “sheets” confirmed the presence of a single crystal of calcite with the $d$-spacings of 2.59, 2.27, 1.72, 1.50 and 1.31 Å, which were assigned to the (1 1 0), (1 1 3), (2 1 1), (1 1 9), (1 2 8) lattice planes of calcite respectively (Figure 5.14 D). Figure 5.15 A shows a high-resolution TEM of a microstructure showing individual crystallites aligned next to each other. The electron diffraction pattern of this microstructure showed its polycrystalline nature with the interatomic distances of 3.45, 2.27, 1.64 and 1.27 Å assigned to the (1 1 2), (2 1 3), (2 1 11) and (2 2 14) lattice planes of vaterite respectively (Figure 5.15 B).

![Figure 5.14](image)

Figure 5.14  
(A) TEM micrograph of CaCO$_3$ particles precipitated from 10 mM Na$_2$CO$_3$ and CaCl$_2$ with 100 µg/ml PSS after a 60 s growth period, the white oval shows the presence of nanoparticles.  
(B) Indexed SAED pattern with calculated $d$-spacings corresponding to vaterite.  
(C) TEM micrograph showing shapeless “sheet”.  
(D) Indexed single crystal SAED pattern with calculated $d$-spacings corresponding to calcite.
After a reaction time of 120 s, the product precipitated in the presence of 100 μg/ml PSS consisted of nanoparticles (indicated by a white oval in Figure 5.16 A), large spherical particles (Figure 5.16 A) and shapeless “sheets” of material (Figure 5.16 C). Electron diffraction patterns originating from large spherical particles consisted of polycrystalline rings with d-spacings of 3.45, 2.86, 2.22 and 1.72 Å assigned to the (1 1 2), (1 0 8), (1 1 9), (1 2 0), (1 0 9), (1 0 5), (0 1 5), (1 0 3), (1 1 3), (0 1 3), (1 0 1), (0 1 1), (1 1 1), (0 0 1) and (1 1 0) planes.
113) lattice planes of vaterite respectively (Figure 5.16 B). Figure 5.16 C depicts an image of a “sheet” of material and the inset shows lattice fringes obtained from the area marked by a white circle. The electron diffraction pattern of this “sheet” showed single crystal character with the \( d \)-spacings of 2.63, 2.29, 1.72 and 1.54 Å being assigned to the (110), (113), (211), (214) lattice planes of calcite respectively (Figure 5.16 D).

Upon leaving all reaction mixtures (at different concentration of PSS) in a glass vial for one day, a mixture of calcite and vaterite was obtained in all cases. At a PSS concentration of 10

![Figure 5.16](image-url)
μg/ml, the calcite obtained was rhombohedral in shape and composed of smaller calcite subunits (Figure 5.17 B). The vaterite particles consisted of small primary nanoparticles, thus giving rise to a porous texture (Figure 5.17 A). At a PSS concentration of 50 μg/ml, the calcite obtained was no longer rhombohedral in shape but possessed one rounded corner. The porous texture of the particle can clearly be seen in Figure 5.18 B. Vaterite obtained in this case was made up of spherical primary nanoparticles (Figure 5.18 A). Upon increasing the concentration of PSS to 100 μg/ml, all corners of calcite particles were rounded. The subunits and the pores of calcite can be seen in the inset of Figure 5.19 B. The vaterite again was made up of spherical primary nanoparticles (Figure 5.19 A).

![Figure 5.17](image)

(A) SEM micrograph of vaterite and (B) calcite precipitated from 10 mM Na₂CO₃ and CaCl₂ with 10 μg/ml PSS after a 1-day growth period.

![Figure 5.18](image)

SEM micrographs of (A) vaterite and (B) calcite precipitated from 10 mM Na₂CO₃ and CaCl₂ with 50 μg/ml PSS after a 1-day growth period.
Figure 5.19  SEM micrograph of (A) vaterite and (B) calcite precipitated from 10 mM Na₂CO₃ and CaCl₂ with 100 µg/ml PSS after a 1-day growth period. The inset shows a high resolution SEM micrograph of calcite.

5.4 Discussion

The synthesis of calcium carbonate in the presence of PSS was performed in pL-volume droplets for the first time. The use of droplet-based microfluidic system allowed precise control of both, the PSS concentration and reaction time. For all concentrations of PSS the sample collected after a reaction time of 2 s consisted only of amorphous calcium carbonate nanoparticles. At a PSS concentration of 10 µg/ml, the number of amorphous particles precipitated after 2 s is significantly lower than at 50 and 100 µg/ml. This supports previous thinking [166] that PSS acts as a nucleating agent by increasing the number of primary amorphous nanoparticles.

After a reaction time of 30 s, the product formed in the presence of PSS at all concentrations, consisted of both small and large amorphous particles as well as areas of densely agglomerated particles. Different sized ACC particles are formed due to different degrees of hydration,[16] different solubilities,[175] different stabilities under electron beam irradiation [151] and different degrees of short-range order.[176] The presence of small amorphous nanoparticles in the reaction mixture at all reaction times, suggests that these nanoparticles act as a feedstock for growing larger nanoparticles. Pouget et al. [174] who studied the initial stages of CaCO₃ formation in the presence of a stearic acid monolayer, also noticed the presence of nanoparticles with sizes of ~30 nm and larger particles at all residence times (up
to 60 min). This observation similarly supports the suggestion that smaller nanoparticles form the feedstock from which the larger crystals grow.[151,177] The authors also found that particles with dimensions less than 70 nm were amorphous. The polycrystalline particles, identified by TEM as vaterite, had a minimum diameter of 70 nm. In another study by Nudelman et al. [154], a single flow microfluidic system was used to synthesise different sizes of ACC nanoparticles by controlling reaction time. The reaction was carried out at 4 °C with poly [(α,β)-DL-aspatic acid] (pAsp) being introduced after 10 s to inhibit further growth and coalescence of ACC. The authors reported that amorphous particles needed to reach a critical size of 100 nm before crystallinity started to develop.[154]

In the current study, the diffraction patterns originating from areas where densely agglomerated amorphous particles were present consisted of amorphous rings together with superimposed spot patterns that can be assigned to the d-spacings of vaterite. This observation indicates that multiple crystalline domains have developed inside the amorphous matrix prior to the formation of a single crystal. A similar situation was observed in the study by Pouget et al.[73] where, while monitoring the early stages of vaterite crystallisation in the presence of NH$_4^+$ ions, they observed the transformation of the amorphous phase into polycrystalline vaterite, followed by transformation into a single crystal. NH$_4^+$ ions stabilised the (00.1) plane of vaterite in the otherwise amorphous matrix, leading to its growth and the subsequent development of hexagonal single crystals. Accordingly, the stabilised crystal phase acts as a template from which crystallinity develops gradually within the particle. To support these findings, the Cryo TEM diffraction pattern of shapeless particles showed the presence of amorphous bands overlaid with a spot pattern.[73]

After a reaction time of 60 s, the product mixture contained small amorphous nanoparticles (feedstock), large spherical nanoparticles with electron diffraction patterns where the d-spacings corresponded to vaterite, and shapeless “sheets”, with single crystal electron diffraction patterns where the d-spacings are characteristic of the calcite polymorph. Large spherical particles were less than 500 nm in diameter and exhibited polycrystalline rings corresponding to the d-spacings of the vaterite polymorph. No amorphous bands were detected. The shapeless “sheets” had single crystal diffraction patterns corresponding to the d-spacings of calcite. In some samples, lattice fringes were detected by HR TEM (Figures 5.7, 5.10 and 5.16), showing the developed crystalline phase. Wang et al. [166] have
previously shown that the addition of PSS to CaCO\textsubscript{3} leads to mesoscale assembly by characterising the finished product as being “porous and more homogeneous”. They proposed that amorphous primary particles form amorphous aggregates and lead to subsequent crystallisation and assembly of mesocrystals. However, the early stages of mesoscale assembly have not until now been studied. Another study showing crystal growth by mesoscale assembly rather than by classical nucleation was performed by Zhou et al. [178]

The high-resolution TEM of the fully formed NH\textsubscript{4}TiOF\textsubscript{3} crystal showed the presence of pores and nanocrystals with critical dimensions of about 25 nm, instead of an atom-level continuous structure. The presence of pores was attributed to the hierarchical structures of mesocrystals. The electron diffraction pattern of those crystals displayed higher crystallinity than that of a conventional polycrystal, which was attributed to the crystallographically ordered alignment of the nanocrystals.[178] The HR TEM of the shapeless “sheets” obtained in the current experiments, also show the presence of porous structures, with darker areas indicating nanocrystals and lighter ones, the pores. The subunit assemblies detected in some samples (Figures 5.10 C and 5.15 A) provide evidence for mesoscale assembly. Finally, the monocristalline nature of the electron diffraction patterns supports the oriented alignment of subunits in mesocrystals and hence mesoscale assembly.

After a reaction time of 120 s, the product mixture obtained had, yet again, small (less than 50 nm in diameter) amorphous nanoparticles present, together with large spherical particles (now more than 500 nm in diameter) and shapeless “sheets”. The large spherical particles had diffraction patterns corresponding to the \(d\)-spacings of vaterite, confirming the development of crystallinity within the particle prior to complete growth. The single crystal electron diffraction of the shapeless “sheets” corresponded to the \(d\)-spacings of calcite.

The final products obtained in all experiments were mixtures of calcite and vaterite. However, the porosity and rough surface of the crystals strongly indicates a shift from ionic growth to a mesoscale assembly. Vaterite obtained at all PSS concentrations was composed of small amorphous spherical nanoparticles. The increase in the concentration of PSS had a more pronounced influence on the structure of calcite. As the concentration of PSS is increased, calcite shifts from being a perfect rhombohedron with calcitic primary units (10 \(\mu\)g/ml PSS) to a distorted rhombohedron with one rounded corner and a porous structure (50
µg/ml PSS) and finally, to a structure where all corners are rounded and are porous, together with subunits that can be clearly seen (100 µg/ml). The effect of PSS is most clearly observed when comparing these results to calcite and vaterite precipitated in its absence, as shown in Chapter 3 (Figures 3.4 – 3.8). Without PSS, the surfaces of calcite and vaterite appear to be continuous and defect-free, and the calcite is a perfect rhombohedron. These findings are supported by a study of the influence of PSS on the precipitation of calcium carbonate by Wang et al.[166] The authors obtained various morphologies ranging from rounded edges and truncated triangles, to concave bent “lens-like” shapes by varying the concentrations of the calcium chloride solutions and the PSS. They noticed an increase in surface roughness and roundness of the corners as the concentration of PSS was increased. As PSS strongly binds to Ca²⁺ ions, the concentration of free Ca²⁺ decreases, thus slowing down the rate of growth of the particles by addition of single ions. This suggested an alteration of the crystallisation mechanism from traditional ionic growth to mesoscale assembly. A mesoscale assembly mechanism was also supported by the evidence that the PSS-Ca complex becomes a primary species in solution, and from the existence of ACC nanoparticles and the presence of primary calcite building units with sizes between 20-30 nm in the final crystals.

5.5 Conclusion

The role of PSS in the precipitation of calcium carbonate has been studied at early reaction stages within pL-volume droplets. The results from these experiments support previous studies, showing that calcium carbonate growth occurs by mesoscale assembly rather than by classical nucleation in the presence of PSS. Moreover, for the first time experiments have probed the early reaction stages under ambient conditions. It can be concluded that the role of PSS is as follows. In the first step, the polymer binds to calcium ions and shifts the reaction mechanism from ionic growth to mesoscale assembly. In the second step, the polymer promotes nucleation, as can be seen from the increased concentration of amorphous nanoparticles as the concentration of PSS increases. These amorphous nanoparticles act as primary building units for the mesocrystals and subsequently crystallise to calcite and vaterite. The droplet microfluidic platform provides a unique way of performing such time-resolved crystallisation studies and can potentially be applied to other crystallisation systems.
Chapter 6

Conclusions
6.1 Conclusions

The work presented herein embodies the first use of droplet-based microfluidic technology to study in depth the crystallisation of calcium carbonate. Currently, there is a significant lack of information on the crystallisation of ACC at ambient temperatures equivalent to those where biomineralisation occurs. Biominerals are known to exhibit outstanding control over crystallinity, morphology, composition and material properties at physiological temperature, pressure and pH ranges. Initial studies focussed on the design and characterisation of microfluidic devices that could controllably and reproducibly form segmented flows and droplets with volumes as small as 100 pL. It was found that a ratio of aqueous flow to carrier flow between 0.2 and 1.25 allowed formation of uniform droplets over a range of flow rates. The average volume of the droplets produced in all experiments in this thesis was 200 pL. PTFE tubing was used to extend the fluidic pathway and therefore prolong residence times.

The developed microfluidic devices were subsequently used to precipitate different polymorphs of calcium carbonate. The concentration of reagents studied ranged from 4 to 12 mM. When precipitating from reagent concentrations less than 4 mM, the reaction proceeded at a very slow rate, not allowing complete crystallisation of particles to occur. When using reagent concentrations above 12 mM a slow build up of precipitate in the channels was observed thus shortening the lifetime of the device and affecting crystallisation conditions. A fine control of polymorph was achieved when using reagent concentrations between 4 and 8 mM, where only calcite or vaterite precipitated respectively. In contrast, experiments performed in bulk solution and in a continuous flow microfluidics, yielded mixtures of both calcite and vaterite polymorphs at all concentrations studied. It was also observed that particles precipitated in droplets were significantly smaller than those precipitated in bulk or continuous flows. For instance, calcite precipitated from 4 mM solutions in droplets had a mean particle size of $2 \pm 0.2$ µm compared to $8.5 \pm 0.9$ µm in a continuous flow and $8.8 \pm 1.5$ µm in bulk. Additionally, droplet-based microfluidic synthesis generated a smaller size distribution of particles due to the absence of the parabolic velocity profiles associated with continuous flows and the unequal mixing of reagents associated with bulk synthesis. In a continuous flow regime, the product collected consisted of particles that have spent varying
amounts of time in a reactor, thus generating a wider size distribution. Under bulk conditions localised concentration gradients across a reaction mixture result in nucleation and growth occurring concurrently and over a greater time period. This results in precipitates having a wide range of sizes.

In further studies the precise control of reaction conditions offered by droplet microfluidic systems enabled the synthesis and isolation of amorphous calcium carbonate, which is extremely difficult to isolate under ambient conditions and without the use of stabilising additives. It was observed that, the droplet-based methodology yielded particles with narrower size distributions than those precipitated from bulk solutions. It was also confirmed that the transformation of the amorphous phase to the crystalline phase occurs via a dissolution-reprecipitation mechanism based of the reduction in the size of the ACC after 120 s. This mechanism is also supported by SEM analysis which shows that vaterite consists of aggregated nanoparticle sub-units. Furthermore, the lack of defined crystal faces on the vaterite sub-units confirms that they have not been formed by controlled precipitation from solution, which usually results in particles with defined crystalline morphology.

The ability to carry out time-resolved studies in microfluidic systems where the product is collected after specific reaction times, enabled investigation of the effect of additives such as, PSS, on the precipitation of calcium carbonate. It was found that PSS binds to calcium ions, thus shifting the mechanism of calcium carbonate growth from ionic growth to mesoscale assembly. Multiple crystalline domains were developed inside the amorphous matrix, leading to the formation of a single crystal. Increasing the amount of PSS in the reaction solution also resulted in increased concentrations of amorphous calcium carbonate, suggesting that PSS also promotes nucleation. These findings are in agreement with previous studies where it was suggested that, in the presence of PSS, calcium carbonate growth occurs by mesoscale assembly rather than by classical nucleation.[167]

**6.2 Increased Operational Functionality**

While the microfluidic system established for the experiments in this thesis was shown to be extremely versatile, it would be advantageous to be able to further increase residence times to
allow a more complete study of crystallisation. The residence times can be increased by prolonging the time droplets spend inside the device and/or incubating offline. The most obvious way of expanding the reaction chamber is to increase the length of PTFE tubing. However, with the build up in pressure, there is a limit to which the tubing can be extended without causing interruption of the flow. This can be overcome by magnifying the dimensions of the channel, thus diverging from a conventional microfluidic device to a droplet reactor. Incubating droplets offline involves storing droplets for an extended period of time outside the device. This could be done by allowing droplets to transfer from the tubing into a vial containing the same oil mixture that was used to generate droplets. Preliminary experiments should be done to test whether droplet breaking or merging occurs.

Furthermore the ability to separate the aqueous phase from the carrier phase online, would significantly improve and simplify the process of sample collection. This has been previously demonstrated on-chip with aqueous droplets and careful consideration and developments should lead to an analogous solution for droplets containing precipitates.[179]

In order to expand the range of reagent concentrations studied in this thesis it is necessary to change the way that the two reagents mix together in order to avoid precipitation on channel walls. The droplet containing aqueous reagent mixtures of known concentrations need to be merged in a controllable and reproducible manner which gives predictable reaction stoichiometries. This has been done with aqueous droplets before and way achieved by electrocoalescence,[108] surface modification,[180] or through the use of passive structures contained within the microfluidic device.[89]

6.3 Other Applications/Future Work

The studies described in this thesis confirm the utility of droplet-based microfluidics in the study of particle crystallisation and thus can be applied to other inorganic crystallisation systems. For instance, calcium phosphate was recognised some 30 years ago as a delivery agent of genetic material to mammalian cells and has since generated a list of methodologies proposed to synthesise a size controlled and stable particles.[181] In regards to expanding the
study of precipitation of calcium carbonate, more additives could be investigated to confirm the growth mechanism. For instance, another polymer, poly(acrylic acid) (PAA) is known to have a similar effect on the crystallisation of calcium carbonate.[182] Hence it would be advantageous to investigate the precipitation of CaCO$_3$ in the presence of PAA in droplet-based microfluidic systems. Finally it would also be of great value to investigate important issues such as variations in crystal growth dynamics with delayed addition of the additives.[183] Such multi-step processes can be accurately carried out in droplet-based microfluidic systems provided that the channel geometry is designed and suitable flow rate for the additive found.
Chapter 7

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