CHAPTER-2

MATERIALS AND METHODS

2.1. MATERIALS AND REAGENTS

2.1.1 MATERIALS

Calcium Chloride and Sodium Carbonate were purchased from Himedia, Nitrilotriacetic acid (disodium salt) and N-(hydroxyethyl)-ethylenediaminetriacetic acid were purchased from Sigma-Aldrich. Ethylenediaminetetraacetic acid (disodium salt) was purchased from Qualigens and Diethylenetriaminepentaacetic acid was purchased from Across Chemicals. All the chemicals were AnalaR grade. Commercial grade 1-Hydroxyethylidene-1,1-Diphosphonic acid (Aquasoft 330) was obtained from Satyajit chemicals, Mumbai. The above chemicals were used as supplied without further purification.

Double distilled water was used for the preparation of all the aqueous solutions. All the experiments were carried out using 0.1 M CaCl$_2$ solution.

2.1.1.1 ETHYLENEDIAMINETETRAACETIC ACID – DISODIUM SALT (EDTA)

EDTA is a versatile chelating agent. EDTA is a hexadentate ligand. It can form four or six bonds with a metal ion, and it chelates/forms complex with both transition-metal ions and main-group ions. Generally EDTA forms 1:1 complexes with metal ions by with 6 ligands [130-132].

Molecular formula : C$_{10}$H$_{14}$N$_{2}$Na$_{2}$O$_{8}$·2H$_{2}$O
Molecular weight : 372.24
EDTA molecule binds a total of six times to a generic central metal and the atoms with the unpaired electrons (the two nitrogen and four oxygen) make these coordinate covalent bonds.

2.1.1.2 NITRILOTRIACETIC ACID - DI SODIUM SALT (NTA)

NTA is an aminotricarboxylic acid and tetradentate ligand. It forms four co-ordination bonds with a metal. It is a synthetic organic metal chelating agent whose metal binding properties are exploited in a broad range of applications i.e., detergent, food, pharmaceutical, cosmetic, metal finishing, photographic, textile and paper industries and it has found widespread use in conditioning reactor cooling water, as an anticorrosive and scale inhibitor and in cleanup operations [130, 133-136].

Molecular formula : $\text{C}_6\text{H}_7\text{NNa}_2\text{NO}_6$

Molecular weight : 235.10

Melting point : $>300 \, ^\circ\text{C}$
The uses of NTA are similar to that of EDTA. However, in contrast to EDTA, NTA is easily biodegradable and is almost completely removed during wastewater treatment. It is used for water softening and as a replacement to sodium and potassium triphosphate in detergents, and cleansers [136]. NTA is a tripodal tetradeutate trianionic ligand [137].

### 2.1.1.3 DIETHYLENETRIAMINEPENTAACETIC ACID (DTPA)

DTPA is a polyamino carboxylic acid consisting of a diethylenetriamine back bone with five carboxymethyl groups. The conjugate base of DTPA has a high affinity for metal cations and it is an octadentate ligand.

- **Molecular formula**: \( \text{C}_{14}\text{H}_{23}\text{N}_{3}\text{O}_{10} \)
- **Molecular weight**: 393.35
- **Melting point**: 220 °C

![Diagram of DTPA molecule]

The formation constants for its complexes are about 100 greater than those for EDTA [26]. As a chelating agent, DTPA wraps around a metal ion by forming up to eight bonds and it binds to number of metals like Zn\(^{2+}\), Mg\(^{2+}\), Mn\(^{2+}\), Co\(^{2+}\), Cu\(^{2+}\), Pb\(^{2+}\) and Ca\(^{2+}\), and various radionuclides [138,139]. DTPA is structurally similar to EDTA except
that it has an extra basic unit of $\text{NCH}_2\text{COO}^-$ and diethylene in place of ethylene in EDTA.

### 2.1.1.4 N-(HYDROXYETHYL)-ETHYLENEDIAMINETRIACETIC ACID (HEDTA)

![HEDTA Structure](image)

- **Molecular formula**: $\text{C}_{10}\text{H}_{18}\text{N}_2\text{O}_7$
- **Molecular weight**: 278.25
- **Melting point**: 212 °C

HEDTA is a hexadentate ligand with three waters of hydration [140,141]. It is being used in industries as a chelating agent in boilers and boiler treatment programmes. However, very little information is available on its chelating ability. To the best of our knowledge the literature survey revealed that no work has been carried out on the chelating properties of HEDTA.

### 2.1.1.5 HYDROXYETHYLIDENE-1,1-DIPHOSPHONIC ACID (HEDP-AQ 330)

Phosphonates have been used as scale and corrosion control additives, as well as dispersants, cleaning agents, chelating agents, and crystal growth modifiers [142]. Phosphonates are advantageous in scale prevention treatments because they have the ability to inhibit different types of scale and are stable over a wide range of conditions, i.e.,
temperatures and pressures [143-145]. Phosphonates are stronger complexing agents than carboxylic acids, with the complexation constants increasing with the number of phosphonate groups per molecule. Di- and trivalent cations such as Ca$^{2+}$, Fe$^{3+}$, and Al$^{3+}$ are precipitated by phosphonates, especially at circum-neutral pH values [146]. The surface chemistry of phosphonates is also important as they are known to adsorb onto silica and clays, barite, cassiterite, aluminum oxides, iron oxides, and calcite [147,148]. In many of these applications, phosphonates are able to react with divalent cations such as calcium to form stable divalent cation phosphonate precipitates. HEDP with Ca$^{2+}$ ions is well-known and this effect is used particularly in water treatment to avoid problems due to strong interaction of phosphonates [149-152].

Molecular formula : $\text{C}_2\text{H}_8\text{O}_7\text{P}_2$

Molecular weight : 206.02

Melting point : 198-199 °C

2.1.2 REAGENTS

2.1.2.1 PREPARATION OF 0.1 M CALCIUM CHLORIDE (CaCl$_2$) SOLUTION

5.549 g of A.R. CaCl$_2$ was dissolved in 500 ml double distilled water.
2.1.2.2 PREPARATION OF 0.1 N SODIUM CARBONATE (\(\text{Na}_2\text{CO}_3\)) SOLUTION

2.65 g of A.R. \(\text{Na}_2\text{CO}_3\) was dissolved in 500 ml double distilled water.

2.1.2.3 PREPARATION OF 0.1 M ETHYLENEDIAMINETETRAACETIC ACID (EDTA) DISODIUM SALT SOLUTION

3.72 g of A.R. EDTA (disodium salt) was dissolved in 100 ml double distilled water.

2.1.2.4 PREPARATION OF 0.1 M NITRILOTRIACETIC ACID (NTA) DISODIUM SALT SOLUTION

2.35 g of A.R. NTA (disodium salt) was dissolved in 100 ml double distilled water.

2.1.2.5 PREPARATION OF 0.1 M N-(HYDROXYETHYL)-ETHYLENEDIAMINETRIACETIC ACID (HEDTA) SOLUTION

2.78 g of HEDTA was dissolved in 100 ml double distilled water.

2.1.2.6 PREPARATION OF 0.1 M DIETHYLENETRIAMINEPENTAACETIC ACID (DTPA) SOLUTION

To 3.93 g of A.R. DTPA in a 100 ml beaker, 5-6 drops of Ammonia (28-30%) was added followed by 50 ml distilled water, stirred well for few minutes and then warmed to dissolve completely and heated to evaporate the excess ammonia. The solution was then quantitatively transferred into a 100 ml volumetric flask and made up to the mark using double distilled water.

2.1.2.7 PREPARATION OF 0.1 M 1-HYDROXYETHYLIDENE-1,1-DIPHOSPHONIC ACID (HEDP (AQ 330)) SOLUTION

2.06 g of HEDP (Aquasoft 330) was dissolved in 100 ml double distilled water.
2.1.2.8 LIST OF ALL CHEMICALS AND REAGENTS USED

<table>
<thead>
<tr>
<th>S. No</th>
<th>Chemical/Reagent</th>
<th>Purity (%)</th>
<th>Supplier</th>
<th>Application/Usage</th>
</tr>
</thead>
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<td>1</td>
<td>Calcium Chloride</td>
<td>99.9</td>
<td>Himedia</td>
<td>Synthesis of samples</td>
</tr>
<tr>
<td>2</td>
<td>Sodium Carbonate</td>
<td>99.9</td>
<td>Himedia</td>
<td>Synthesis of samples</td>
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<tr>
<td>3</td>
<td>EDTA</td>
<td>99.9</td>
<td>Qualigens</td>
<td>Chelating agent</td>
</tr>
<tr>
<td>4</td>
<td>NTA</td>
<td>99.9</td>
<td>Sigma-Aldrich</td>
<td>Chelating agent</td>
</tr>
<tr>
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<td>HEDTA</td>
<td>99.9</td>
<td>Sigma-Aldrich</td>
<td>Chelating agent</td>
</tr>
<tr>
<td>6</td>
<td>DTPA</td>
<td>99.9</td>
<td>Across Chemicals</td>
<td>Chelating agent</td>
</tr>
<tr>
<td>7</td>
<td>HEDP (Aquasoft 330)</td>
<td>Commercial grade</td>
<td>Satyajit Chemicals</td>
<td>Chelating agent</td>
</tr>
<tr>
<td>8</td>
<td>AMMONIA</td>
<td>99.9</td>
<td>Sigma-Aldrich</td>
<td>To adjust the pH</td>
</tr>
</tbody>
</table>

2.2 SYNTHESIS OF CaCO₃ SAMPLES

2.2.1 SYNTHESIS OF CaCO₃ SAMPLES BELOW 100 °C

In a typical synthesis 50 ml 0.1M CaCl₂ and 20ml of 0.1 M sequestrant solution (in the case of blended systems, a mixtures of 10 ml of each chelating agent solution) were taken in a round bottom flask and heated to 60 °C in a rotamantle with constant stirring. After attaining the temperature Na₂CO₃ was added slowly drop by drop till the precipitation begin. The contents were then aged for 10-12 h at the same temperature. Finally the precipitate was filtered through Whatman (No 40) filter paper, washed thrice with distilled water and then dried at 45 °C in an oven for 3-4 h. The experiment was carried out at 80 °C.

2.2.2. SYNTHESIS OF CaCO₃ SAMPLES AT 100 °C AND ABOVE

50 ml 0.1 M CaCl₂ and 20 ml of 0.1M sequestrant (in the case of blended systems, a mixtures of 10 ml of each chelating agent solution) and 50 - 75 ml 0.1 N Na₂CO₃ were consecutively added in a Buchner
flask, closed tightly with a glass lid to avoid any contamination and kept inside the autoclave (Reactions above 130 °C were carried out in Teflon bottles inside a hydrothermal bomb, which consists of Teflon bottle having volume of about 50 ml with tight lid of about 5 mm). The autoclave was then heated to 10 °C below the reaction temperature for about 2 - 4 h and the precipitate formed if any was filtered using Whatman-40 filter paper. This was done to remove any precipitate formed below the reaction temperature. The clear solution was kept again in the autoclave and heated to reaction temperature and aged for 24 - 72 h and resultant precipitate was washed with double distilled water, filtered using Whatman-40 filter paper and then dried at 45 °C in an oven for 2 - 3 h. The details regarding experimental parameters are provided in Table 2.1 (at the end of this chapter).

2.3. EQUIPMENTS USED FOR SYNTHESIS OF CaCO₃

2.3.1 ROTAMANTLE

A rotamantle with temperature controller manufactured by Remi laboratory instruments, India was used for experiments below 100 °C.

2.3.2 PROGRAMMABLE AUTOCLAVE

A programmable autoclave with digital temperature and pressure controller manufactured by Everflow Scientific Instruments, Chennai was used for synthesis at 100 and 130 °C.
2.3.3 HYDRO THERMAL AUTOCLAVE

A programmable autoclave with digital temperature and pressure indicator and automatic controller, manufactured by Thermocon Instruments Pvt. Ltd., Bangalore was used for synthesis above 130 °C and the reactions were carried out in Teflon bottles inside a hydrothermal bomb.

2.4 INSTRUMENTS USED FOR CHARACTERIZATION OF CaCO₃
POLYMORPHS

2.4.1 FOURIER TRANSFORM INFRARED SPECTROSCOPY

It is well known that different crystal forms of CaCO₃ show different bands in FT-IR spectrum [153,154]. FT-IR spectra were taken after KBr pelletization, in the range 500 to 4000 cm⁻¹ using Avatar-330 FT-IR and JASCO-5300 FT-IR instruments. Reference bands around 700-750, 874-878, 1435-1418 cm⁻¹ are assigned to $ν_4$ in-plane bending, $ν_2$ out-of-plane bending, and $ν_3$ asymmetric stretching modes of CO$_3^{2-}$ respectively. The characteristic carbonate absorption bands assigned for different polymorphs are; 700 and 712 cm⁻¹ ($ν_4$), 856 cm⁻¹ ($ν_2$), 1082 cm⁻¹ ($ν_1$), 1475 cm⁻¹ ($ν_3$) for aragonite, 711 cm⁻¹ ($ν_4$), 878 cm⁻¹ ($ν_2$), 1079 cm⁻¹ ($ν_1$) for calcite and 745 cm⁻¹ ($ν_4$), 876 cm⁻¹ ($ν_2$), 1087 cm⁻¹ ($ν_1$), 1450 cm⁻¹ ($ν_3$) for vaterite [155-158]. Wherever there is a strong overlapping of bands between calcite and aragonite, the spectra were deconvoluted.

2.4.2 POWDER X-RAY DIFFRACTION SPECTROSCOPY

Powder X-ray diffraction (XRD) is a very important tool for identification, confirmation and quantitative estimation of different
crystalline forms. The X-ray diffraction (XRD) patterns were recorded on a Bruker D8 Advanced XRD diffractometer with Cu Kα radiation at λ=1.5406 Å with the step size of -0.091063. DIFFRACplus EVA software was used for data evaluation. Presence of different crystalline polymorphs were confirmed by appearance of peaks at (2θ values) ~ 29.4° (1 0 4), 26.2° (1 1 1) and 32.7° (0 2 2), (1 1 2) for calcite, aragonite and vaterite respectively and were compared with standard Joint Committee on Powder Diffraction Standards (JCPDS) data. The relative contents of the different polymorphic forms were calculated from the XRD data using the following equations 1-3 [159].

\[ X_A = \frac{3.157 \times I_A^{221}}{I_c^{104} + 3.157 \times I_A^{221} + 7.691 \times I_v^{110}} \to 1 \]

\[ X_c = \frac{I_c^{104} \times X_A}{3.157 \times I_A^{221}} \to 2 \]

\[ X_v = 1 - X_A - X_c \to 3 \]

here the subscripts C, V and A denote calcite, vaterite and aragonite, respectively. X is the fraction of phase component, I is the intensity of the corresponding XRD peak.

2.4.3 RAMAN SPECTROSCOPY

Since the structures of aragonite and calcite are spectroscopically distinct and well documented, the structural differences between different polymorphs of CaCO₃ can be easily distinguished by Raman spectroscopy [160,161]. Raman spectra of the samples were obtained with the WI Tec Confocal Raman Microscope alpha 300 R Raman spectrometer excited by laser line having a wavelength of 488 nm,
provided by an argon laser. The laser beam power was amounted to 2 mW and the acquisition time was 1 min.

The vibrational spectra of carbonates can be divided in two regions. The region below 400 cm\(^{-1}\) contains bands that are due to motions comprising the complete unit cell, which is usually referred to as the lattice modes. The second region includes bands above 400 cm\(^{-1}\) which are caused by the internal modes of the molecular carbonate ion [162,163]. In-plane bending (\(\nu_4\)) bands at 711 cm\(^{-1}\), 701 cm\(^{-1}\) and 745 cm\(^{-1}\) were assigned to calcite, aragonite and vaterite respectively. Symmetric stretching band at 1090 cm\(^{-1}\) was assigned to vaterite whereas the band at 1085 to 1087 cm\(^{-1}\) were assigned for both calcite and aragonite depending upon the presence of other confirmatory bands i.e., in-plane bending, lattice modes, and asymmetric stretching. Characteristic Raman bands of calcite, aragonite and vaterite are given in the table below.

<table>
<thead>
<tr>
<th>Polymorph</th>
<th>Lattice modes (cm(^{-1}))</th>
<th>(\nu_4) In-plane bending (cm(^{-1}))</th>
<th>(\nu_2) Out-of-plane bending (cm(^{-1}))</th>
<th>(\nu_1) Symmetric stretching (cm(^{-1}))</th>
<th>(\nu_3) Asymmetric stretching (cm(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcite</td>
<td>151,154, 281</td>
<td>711,712</td>
<td>--</td>
<td>1085-1087</td>
<td>1434, 1435</td>
</tr>
<tr>
<td>Aragonite</td>
<td>217,222, 287</td>
<td>701-703, 712,717</td>
<td>--</td>
<td>1085-1087</td>
<td>1464, 1466</td>
</tr>
<tr>
<td>Vaterite</td>
<td>210-212, 301-304, 325-334</td>
<td>745-750</td>
<td>877, 881</td>
<td>1090, 1091</td>
<td>1441, 1484</td>
</tr>
</tbody>
</table>

Table 2.2 Characteristic Raman bands (wave numbers in cm\(^{-1}\)) of calcite, aragonite and vaterite [160]
2.4.4 MORPHOLOGICAL STUDIES USING SCANNING ELECTRON MICROSCOPE

Microscopic morphological images were taken using Philips XL30 - ESEM and Hitachi Science systems S-3400N Scanning Electron Microscopes (SEM) having accelerating voltage of 10-30 kV (in High Vacuum Mode) with Secondary electron Image Resolution: \(~ 3.0 \text{ nm}\).

Field emission scanning electron microscope images were taken using CARL ZESIS (FE-SEM) with in lens detector to take high magnified/resolution images of selected CaCO\(_3\) samples and to observe the nano structure in a better way. For both SEM/FE-SEM, samples were mounted onto a circular aluminum stub with carbon adhesive pads. In order to have a uniform distribution, the samples were applied on glass beads using dispersion method, dried and then affixed on to the carbon adhesive pad. The samples were coated with gold prior to imaging.

2.4.5 TRANSMISSION ELECTRON MICROSCOPY (TEM) AND ENERGY DISPERSIVE X-RAY SPECTROSCOPY (EDAX)

To further analyze the morphology and nano-structure of the CaCO\(_3\) samples, Transmission electron microscope (TEM) imaging and selected area electron diffraction studies were carried out on a Tecnai G2 FEI F12 TEM at an accelerating voltage of 200 kV. The sample was taken in vial and dispersed in water. A drop of the this dispersion was taken in micro pipette and placed on carbon-coated copper grid (Icon analytical, mesh 200, type B), kept upon Whatman filter paper in
petri dish. The sample was then dried in a vacuum desiccator for 4 hours and then analyzed. Energy dispersive X-ray spectroscopy was performed by using TEAM EDAX- appollo XL TLS (AMETEK). EDAX results were used for confirmation of the compound and atomic percentages of CaCO$_3$ in nano structures.

2.4.6 pH METER

The pH measurements were made by using digital Elico pH meter model LI-120.

2.4.7 LIST OF ALL EQUIPMENTS USED

<table>
<thead>
<tr>
<th>S. No</th>
<th>EQUIPMENT</th>
<th>Application/Usage</th>
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<td>1</td>
<td>Rotamantle</td>
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