CHAPTER 3

EXPERIMENTAL METHODS

3.1 SYNTHESIS OF HA POWDER

3.1.1 Processing of Sea Shells

Nanocrystalline HA was synthesized by wet chemical reaction between sea shell powder-calcium oxide (CaO) and phosphoric acid (H$_3$PO$_4$).

Sea shells obtained from the South Indian coastline of Trivandrum, Kerala, are the source material for this process. The sea shells (CaCO$_3$) which were mostly of the molluscan marine species *Mytilus sp* (Brown Mussel) were harvested before they were cleaned, deproteinized and mixed with coke. These were dumped into a mud pit and the mixture was heated by hot air derived out of burning coke, through a blower arrangement available at the side of the pit. Water was occasionally poured on top of the mixture to prevent charring of the sea shells. The sea shells were heated along with the coke for 4 hours, and they were stirred manually to ensure uniform heating. The temperature attained was around 900°C. Then, the already derived amorphous shells of CaO obtained from previous heating of sea shells were laid on top of the heated sea shells, and the mixture was further heated for half an hour. The blower was then stopped, and the mixture was left in the pit for 2 days (48 hours). The heated amorphous shells of Calcium Oxide were removed from the pit, and separated from the unburnt sea shells.
The following is the chemical reaction involved:

\[
\begin{align*}
\text{CaCO}_3 \quad &\rightarrow \quad \text{CaO} + \text{CO}_2 \\
\text{(Sea shell powder)} \quad &\quad (3.1)
\end{align*}
\]

Carbon dioxide gas (CO₂) was released upon heating sea shells (CaCO₃), producing soft amorphous Calcium oxide (CaO), which was powdered using a mortar and pestle, and this sea shell powder had a particle size of 106 microns.

### 3.1.2 Synthesis of HA without and with Microwave Irradiation

Two experiments were conducted by wet chemical synthesis, using powdered seashells (CaO) as described in sections 3.1.2.1 and 3.1.2.2:

#### 3.1.2.1 Wet Chemical Synthesis without Microwave Irradiation

Calcium oxide (2.8 g of CaO dissolved in 200 ml of distilled water) was used to prepare 0.25M solution of calcium hydroxide, to which 0.15M of phosphoric acid (1.7 ml of phosphoric acid dissolved in 200 ml of distilled water) was added drop-wise at the rate of 1ml /minute, with continuous stirring using a magnetic stirrer at room temperature as shown in Figure 3.1. The above combination gives a Ca:P ratio of 1.67 for the reagents. The related chemical reactions are given below:

\[
\begin{align*}
\text{CaO} + \text{H}_2\text{O} \quad &\rightarrow \quad \text{Ca(OH)}_2 \\
\text{(Hydroxyapatite)} \quad &\quad (3.2)
\end{align*}
\]

\[
\begin{align*}
10 \text{Ca(OH)}_2 + 6 \text{H}_3\text{PO}_4 \quad &\rightarrow \quad \text{Ca}_{10} (\text{PO}_4)_6 (\text{OH})_2 + 18 \text{H}_2\text{O} \\
\text{(Hydroxyapatite)} \quad &\quad (3.3)
\end{align*}
\]

Continuous stirring to complete the chemical reaction resulted in a gelatinous precipitate, and an ammonia solution was added drop wise to
maintain the pH at 10 to avoid the formation of calcium deficient apatites. The solution was allowed to age for 24 hours, and then filtered to separate the precipitate which was washed with distilled water three times. Then, the precipitate obtained was dried in a furnace at 110°C for 6 hours. The dry sample obtained was powdered well using a mortar and pestle.

![Figure 3.1 Magnetic stirrer for the synthesis of HA](image)

3.1.2.2 Wet Chemical Synthesis with Microwave Irradiation

Microwave irradiation of the reactant solution and drying of the resultant precipitate were done using a domestic microwave oven (LG, 2450 MHz, 800 W Power).

Calcium oxide (2.8 g of CaO dissolved in 200 ml of distilled water) was used to prepare 0.25M solution of calcium hydroxide, to which 0.15M of phosphoric acid (1.7 ml of phosphoric acid dissolved in 200 ml of distilled water) was added drop-wise at the rate of 1ml /minute, with continuous stirring using a magnetic stirrer at room temperature, maintaining the same Ca:P ratio of 1.67 for the reagents. Continuous stirring was done until the
reaction was complete, and the pH was maintained at 10 by the drop-wise addition of the ammonia solution. The solution was then subjected to microwave irradiation at 100 % power (800 W) for 10 minutes. Then, the solution was filtered to obtain the precipitate, which was washed with distilled water and dried in the microwave oven for 15 minutes at 60% power (480W), to remove the water particles completely. The dried sample was powdered well, using a mortar and pestle.

3.1.2.3 Reaction Calculations for the synthesis of HA

Molarity (mol/L, molar, M) denotes the number of moles of a given substance per litre of solution.

\[
\text{Molarity} = \frac{\text{Moles of Solute}}{\text{Litres of Solution}} \quad (3.4)
\]

Number of moles \( = \frac{\text{Weight of Substance}}{\text{Molecular Weight}} \quad (3.5)\)

\[
\text{Weight of substance required for the reaction} = \frac{x \times \text{Molarity} \times \text{Mol.Wt} \times \text{y Lit.}}{1000} \quad (3.6)
\]

For the above experiment, 0.25M of Ca(OH)\(_2\) and 0.15M of H\(_3\)PO\(_4\) were used, which were calculated as follows:

Molecular Weight of CaO \( = 56 \text{ g/mol} \)

\[
\text{Quantity of CaO required} = \frac{0.25 \times 56 \times 200}{1000} = 2.8 \text{ g in 200 ml of Water}
\]
Molecular Weight of Phosphoric acid = 98 g/mol

Quantity of Phosphoric Acid required = \frac{0.15 \times 98 \times 200}{1000} = 2.94 \text{ g in 200 ml of Water}

Density of Phosphoric Acid = 1.75 \text{ g/ml}

Quantity of Phosphoric Acid required = \frac{2.94}{1.75} \approx 1.7 \text{ ml in 200 ml water}

3.1.3 Synthesis of HA by Varying the Concentration of the Reactants

Two sets of experiments – first by furnace drying (FH) and the second by microwave irradiation and drying (MH) were carried out; in the first experiment, the HA obtained was furnace dried, and in the second experiment, the reactant solution was irradiated with the microwave, and the HA was dried in the microwave oven. In each set of experiments, the concentrations of the reactants were decreased gradually.

3.1.3.1 Synthesis of HA by furnace drying (FH)

Wet chemical synthesis was adopted to synthesize HA from sea shell powder as per the procedure enumerated in section 3.1.2.1. Phosphoric acid was added drop-wise at the rate of 3ml/minute to a solution of calcium hydroxide, maintaining a Ca:P ratio of 1.67. The pH for the reaction was maintained at 10 by the drop-wise addition of ammonia solution.

As per the reaction equations (2) and (3), HA was formed during the first set of experiments (FH1-FH4). The solution was allowed to age for 24 hours after precipitation, and then filtered to separate the precipitate which
was washed with distilled water three times. The precipitate obtained was
dried in the atmosphere for 18 hours, and then in an electric furnace at 80°C
for 8 hours. The dried samples were powdered well, using a mortar and pestle.

3.1.3.2 Synthesis of HA by microwave irradiation and microwave
drying (MH)

In the second set of experiments (MH1-MH4), after the formation
of the gelatinous precipitate, the reactant solution was subjected to microwave
irradiation in a domestic microwave oven (LG, 2450 MHz, 800 W Power) at
800 W (100% power) for 10 minutes. The solution was filtered to obtain the
precipitate, which was washed with distilled water and dried in the
atmosphere for 18 hours. Then, the precipitate was dried in the microwave
oven for 25 minutes at 60% power (480W), to remove the water particles
completely.

The concentration of the reactants for both FH1-FH4 and MH1-
MH4 are given in Table 3.1.

Table 3.1 Concentration of the reactants for the experiments FH and MH*

<table>
<thead>
<tr>
<th>EXPT.</th>
<th>Ca(OH)₂ (M)</th>
<th>H₃PO₄ (M)</th>
<th>Wt. of CaO (Sea shell powder) added to 200ml of water (g)</th>
<th>Volume of H₃PO₄ (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FH1 &amp;MH1</td>
<td>4</td>
<td>2.4</td>
<td>44.8</td>
<td>26.88</td>
</tr>
<tr>
<td>FH2 &amp;MH2</td>
<td>2</td>
<td>1.2</td>
<td>22.4</td>
<td>13.44</td>
</tr>
<tr>
<td>FH3 &amp;MH3</td>
<td>1</td>
<td>0.6</td>
<td>11.2</td>
<td>6.72</td>
</tr>
<tr>
<td>FH4 &amp;MH4</td>
<td>0.5</td>
<td>0.3</td>
<td>5.6</td>
<td>3.36</td>
</tr>
</tbody>
</table>

* Ca: P ratio maintained = 1.67
3.2 THERMAL STABILITY STUDIES ON SYNTHESIZED HA

The HA powder obtained through the experiment MH1, was subjected to thermal studies, as the thermal degradation of HA prepared from sea shells is not known. For this, the powder was divided into different parts of 1 g each and heated in the furnace and domestic microwave oven, which was used in the convection combination mode. The experimental parameters adopted for heating HA are given below in sections 3.2.1 and 3.2.2:

3.2.1 Experimental Parameters for Furnace Heating

Weight of the sample : 1 g
Temperatures maintained : 250°C, 500°C, 750°C and 1000°C
Soaking temperature : 2 hours
Cooling method : cooling in still air

3.2.2 Experimental Parameters for Microwave Heating

Weight of the sample : 1 g
Temperature maintained : 180°C, 200°C, 220°C, 230°C
Power : 540W (60%)
Soaking temperature : 20 minutes
Cooling method : cooling in oven for 1 hour

3.3 ELECTROSPUN COATING OF POLYSULFONE (PSU)-HA COMPOSITE ON Ti-6Al-4V ALLOY

3.3.1 Preparation and Testing of Ti-6Al-4V Alloy Substrate

The Titanium alloy Ti-6Al-4V sheet of 2mm thickness was procured from South Asia Metal and Alloys, Mumbai, India. The Titanium
alloy sheet was characterized to determine the alloy composition, microstructure, microhardness and mechanical properties.

The sheet was cut into small pieces of size 30mmx10mmx2mm, which were buffed to mirror-finish and sand blasted to an average surface roughness of 1 micron. Surface roughness values were measured by a surface roughness measuring tester SJ-210 (MITUTOYO) at a traverse speed of 0.5mm/s. Table 3.2 indicates the chemical composition of the procured titanium alloy as tested in the lab.

**Table 3.2 Chemical Composition of Ti-6Al-4V alloy**

<table>
<thead>
<tr>
<th>Elements</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Copper</td>
<td>0.031</td>
</tr>
<tr>
<td>Silicon</td>
<td>0.011</td>
</tr>
<tr>
<td>Manganese</td>
<td>0.0070</td>
</tr>
<tr>
<td>Chromium</td>
<td>0.003</td>
</tr>
<tr>
<td>Nickel</td>
<td>0.0005</td>
</tr>
<tr>
<td>Aluminium</td>
<td>5.912</td>
</tr>
<tr>
<td>Chromium</td>
<td>0.0010</td>
</tr>
<tr>
<td>Titanium</td>
<td>89.141</td>
</tr>
<tr>
<td>vanadium</td>
<td>4.715</td>
</tr>
<tr>
<td>Iron</td>
<td>0.168</td>
</tr>
</tbody>
</table>
Figure 3.2  (a) Tensile tests conducted on the specimens (1) and (2)  
(b) Results of the tensile tests

Figure 3.2 (a) and (b) indicate the tensile test results for specimens (1) and (2) and the mechanical properties correlated with the standard Ti-6Al-4V alloy.
Figure 3.3 indicates the three specimens for Charpy tests and the average strain energy of the procured Ti-6Al-4V alloy was found to be 24 Joules.

The average microvicker’s hardness value of Ti-6Al-4V was 305 H.V at 0.5 kg load.

The above results confirmed that the specimen procured for use as substrate was Ti-6Al-4V alloy.

3.3.2 **Electrospinning of PSU-HA Solution**

Polysulfone (PSU) in the form of pellets purchased from SIGMA-ALDRICH, USA ($M_w =35,000$ and $M_n =16,000$), was mixed separately with a solvent N-Methyl Pyrrolidinone (NMP) at different concentrations (12%, 15%. and 20% by weights of solute). The solution was stirred for 3 hrs in a magnetic stirrer and during the process, a temperature of 40°C was maintained during the first one hour, to ensure effective dissolution of PSU in NMP.

The electro spinning equipment consisted of a 2 ml syringe (Dispovan-needle size 0.55mmx25mm), with the needle positioned horizontally on a clamp as shown in Figure 3.4. The different process parameters of electrospinning, namely, the flow rate of the polymer solution, DC voltage applied to the needle, the distance (d) between the needle and the substrate, and the concentration (C) of the polymer solution were varied as shown in Table 3.2, to determine the optimized parameters for the formation of nanofibres.

HA powder in different proportions (1%, 2% and 3% by weight) obtained from the experiment MH1 was separately dispersed in acetone and
stirred for 30 minutes at room temperature to minimize the agglomeration of HA, and then it was mixed with PSU and ultrasonicated for one hour in the composite solution.

Table 3.3  Effect of various process conditions on the electrospinning of PSU-HA Composite solution

<table>
<thead>
<tr>
<th>Flow rate of the polymer solution</th>
<th>Applied DC Voltage</th>
<th>Distances between the needle and the substrate (d)</th>
<th>Concentration of the polymer solution ( % by weights of solute) (C)</th>
<th>Observation</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1 ml/h</td>
<td>22 kV</td>
<td>15 cm</td>
<td>12%</td>
<td>Thin fibres dominated by beads due to spraying of droplets formed on the substrate.</td>
</tr>
<tr>
<td>0.1 ml/h</td>
<td>22 kV</td>
<td>12 cm</td>
<td>20%</td>
<td>Almost beadless nano fibres deposited on the substrate.</td>
</tr>
<tr>
<td>0.1 ml/h</td>
<td>22 kV</td>
<td>10 cm and 15 cm</td>
<td>20%</td>
<td>Nano fibres of PSU with HA embedded within the fibres were deposited on the substrate.</td>
</tr>
</tbody>
</table>
Upon conducting various trials, it was found that the formation of nanofibres occurred smoothly under the conditions as given in the Table 3.3. The substrates were Ti-6Al-4V specimen pieces of size 30mmx10mmx2mm of roughness Ra~1µm. At fixed conditions of a feed rate of 0.1ml/hour and DC voltage of 22kV, the concentrations of the polymer solution and the distance between the needle and substrate were varied, to study the morphology of the electrospun nanofibres.

Figure 3.4  Electro spinning set up along with the High Voltage DC Source

The substrates electro spin coated with the PSU-HA composite in concentrations of 1 %, 2% and 3 % of HA by weight and C=20%, d=12cm were immersed in a simulated body fluid (SBF) for 4 days, 7 days and 14 days respectively. The SBF was prepared as per the protocol reported by Kokubo et al (1990), as shown in Table 3.4.

The Ti-6Al-4V sheet of 2 mm thickness was cut into small pieces of 5mmx5mm, and immersed in a simulated body fluid in 20ml containers as
shown in Figure 3.5, and maintained at a temperature of 37°C. At the end of
the required time, the specimens were taken out, washed with distilled water
three times and dried at 40°C for 6 hours, before the required
characterizations were done.

Table 3.4 Ion concentrations of the simulated body fluid and human
blood plasma (Kokubo et al 1990)

<table>
<thead>
<tr>
<th>Ion</th>
<th>Concentration (mmol/dm$^3$)</th>
<th>Simulated Body Fluid</th>
<th>Human Blood Plasma</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na$^+$</td>
<td>142.0</td>
<td></td>
<td>142.0</td>
</tr>
<tr>
<td>K$^+$</td>
<td>5.0</td>
<td></td>
<td>5.0</td>
</tr>
<tr>
<td>Mg$^{2+}$</td>
<td>1.5</td>
<td></td>
<td>1.5</td>
</tr>
<tr>
<td>Ca$^{2+}$</td>
<td>2.5</td>
<td></td>
<td>2.5</td>
</tr>
<tr>
<td>Cl$^-$</td>
<td>147.8</td>
<td></td>
<td>103.0</td>
</tr>
<tr>
<td>HCO$_3^-$</td>
<td>4.2</td>
<td></td>
<td>27.0</td>
</tr>
<tr>
<td>HPO$_4^{2-}$</td>
<td>1.0</td>
<td></td>
<td>1.0</td>
</tr>
<tr>
<td>SO$_4^{2-}$</td>
<td>0.5</td>
<td></td>
<td>0.5</td>
</tr>
</tbody>
</table>

Figure 3.5  Bioactivity tests on the coated specimens immersed in a
simulated body fluid