

**Synthesis Of Hydroxyapatite Nanocomposite  
Reinforced With 2D Boron Nitride For Load Bearing  
Orthopedic Applications**



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**This report is submitted as a FYP thesis in partial fulfillment of the  
requirement for the degree of**

**(BE in Materials Engineering)**

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**May 2018**

## Certificate

This is to certify that the work in this dissertation/report has been carried out by; Mr. Hamza Javed Alvi, Mr. Asad-ur-Rehman Qureshi and Mr. Muhammad Mohsin Saeed, completed under my supervision in Nano synthesis, Microscopy, Surface Treatment and Chemical Analysis Lab at the School of Chemical and Materials Engineering, National University of Sciences and Technology, NUST, H-12, Islamabad.

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## **Dedication**

We dedicate this thesis to our parents, our teachers, our siblings and our friends; People whose constant guidance and inspiration paved our path and who supported us in our best and worst.

## **Acknowledgement**

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## **Abstract**

Hydroxyapatite  $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$  is structurally and chemically similar to mineral phase of natural bone and is used in bone implants. In this project, bioactive and biocompatible hydroxyapatite was synthesized by using eggshells as a calcium precursor. Microwaves were used instead of the conventional heating method which greatly reduces the cost and time required to produce it. Hydroxyapatite is largely used in bone implants, but its low flexural strength limits its use where implants undergo flexural stress. The aim of this project was to remove this deficiency of hydroxyapatite by reinforcing it with 2D boron nitride. Thus nanocomposite will gain its strength from 2D boron nitride and maintain the biocompatibility due to hydroxyapatite. It will ensure the use of hydroxyapatite in implants where flexural stress is involved such as total hip replacement. The nanocomposite formed in this way can either be used in bone scaffold or as a coating on metal implants providing the required mechanical properties with added benefits of rapid synthesis and low-cost processing.

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# List of Abbreviations

HA - Hydroxyapatite

BN – Boron Nitride

XRD – X-Ray Diffraction

SEM – Scanning Electron Microscopy

FTIR – Fourier Transform Infrared

AFM – Atomic Force Microscope

# Chapter 1

## Introduction

The human skeleton, is a vital part of a working human body. It gives the human body a structure, it protects vital organs and it grows with us as we age. However, even if one has not been in an accident that causes certain injuries that require a bone replacement, there comes a point that this structure begins to deteriorate over time. This is especially profound in the later stages of our life cycle as we age.

Thus, to solve this issue, a material is required that can repair or replace the affected bone. This material should be bio-compatible, it should have the physical ability to carry out the same tasks as regular bone and it should be flexible enough to take a specific shape of the bone to be replaced, for it to be deemed feasible.

This is where we turn to Hydroxyapatite (HA). While also present in the bone, Hydroxyapatite is a biocompatible material which can also be artificially synthesized and is a suitable replacement for bone in the human skeleton.

Hydroxyapatite is a calcium phosphate which is already found in major proportions within our bones. What is important about HA is that it is a bioactive material. Our aim with this project, is to produce HA by using egg shells as a calcium source. Microwaves are adopted over conventional heating methods for refluxing as they provide faster and economic route for synthesis.

The next and main step of our project, is to form a HA nanocomposite reinforced with 2D boron nitride. 2D BN is selected for its excellent and promising mechanical properties. We aim to remove the mechanical limitations of HA by incorporating 2D BN to make it more suitable for load bearing orthopedic application such as bone implant and total hip replacement. If the desired properties are obtained, the scope of this project can be extended to dental implants as well. [1]

# Chapter 2

## Literature Review

### **What are Biomaterials?**

Biomaterials are as the name suggests, materials (whether organic or synthetic) that are made with the specific function to allow them to be placed within the human body for purposes such as to provide repair or replacement of a missing tissue and can also extend towards providing similar benefits for bone and cartilage.

The main reason why biomaterials are specified into a category, is because not every material is compatible to be placed inside the human body. Many of them can cause reactions when they come into contact with either the bone or tissue itself along with the many other bodily fluids present. These reactions are dangerous and can lead to the formation of toxic substances within the body which is why most materials are ill suited towards this purpose. However, this is where biomaterials are required. Synthesized or naturally occurring biomaterials do not pose this threat. These are completely inert and some can even allow the formation and growth of bone around them which is a property that is termed as bioactivity.

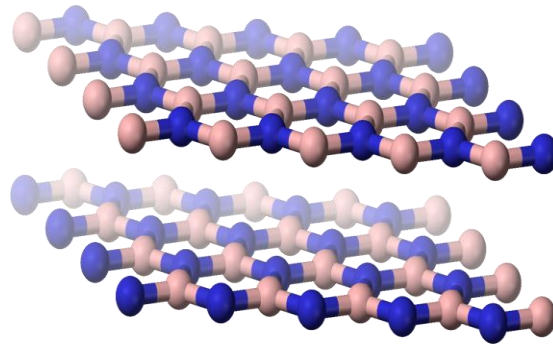
A various range of biomaterials have been synthesized to this day. Most of them are already in use by the orthopedic and orthodontic professions, while the rest are under testing and improvement by the R&D departments of pharmaceutical industries. [2]

Biomaterials are not just simply inserted into the body. Instead, there is a variety of methods and shapes of biomaterials that gives them a specific purpose. For example:

- Stents – biocompatible stents that do not react with the blood flow in and out of the heart
- Filling material – biocompatible replacement for the enamel lining on the teeth that protects the nerves underneath from exposure.
- Titanium rods and screws – used to treat deformed or broken bones
- Joint replacement
- Various implants – to replace or repair degraded bones and tissue
- Surgical Mesh
- Valves
- Synthetic organs – Such as a pacemaker
- Ocular Lenses
- And many others

### **What are 2-D nanomaterials?**

2D nanomaterials is the class of nanomaterials whose structures are only one to few atoms thick. As the name suggests, they have 2 dimensional crystal structure and are present in the form of sheets and layers. Despite being so thin and light in weight, they exhibit remarkable strength and other useful properties such as heat and electrical conductivity and flexibility. They are basically two dimensional allotropes of various elements and compounds such as the Graphene is a two dimensional allotrope of graphite. Unlike steel, their discovery is considered recent so a lot of research is being done in this field. Two dimensional nanomaterials have the potential to revolutionize the field of electronics such as semiconductors, transistors and solar cells, sensors, composites etc.



*Figure 1 - Graphic representation of 2D materials*

Graphene, Boron Nitride, Molybdenum disulphide, Silicene are all various two dimensional nanomaterials. Graphene is the most important 2D nanomaterial of this time. Despite being single atom layer, it is 300 times stronger than steel. It is considered the hardest and strongest material in the world and has many useful properties like super conductivity and flexibility. These properties make it the most liked 2D nanomaterial and a lot of research is being conducted on it to take the maximum benefit of it. Since it is an allotrope of carbon, it is black in color which makes it unsuitable for our project applications so we shifted to the second most famous and useful 2D nanomaterials that is boron nitride. [3] [4] [5]

### **2D Boron Nitride:**

2D boron nitride is a crystalline form of hexagonal boron nitride. It has hexagonal crystal structure and is few atoms thick. It is structurally similar to Graphene but has different electronic and chemical properties. It is electrically insulator and has a band gap of ~5.9 eV. Boron nitride exists in the form of hexagonal, cubic and wurtzite forms. Hexagonal form is the most stable and superior form and is the one used in our project as well. 2D BN can be formed in the form of nanosheets, nanotubes or nanomeshes depending upon the applications and required properties.

## **Properties of 2D BN Nano-sheets:**

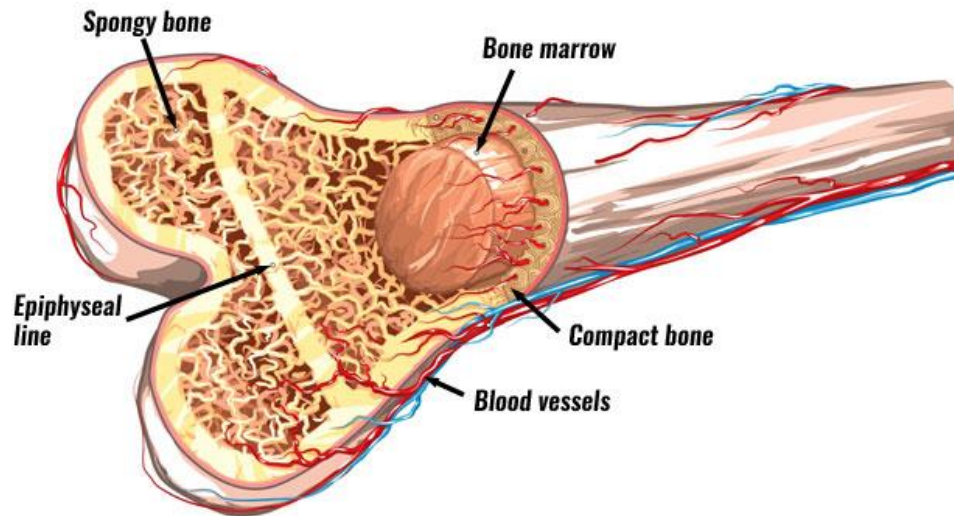
As stated earlier, Graphene is the most used and studied 2D nanomaterial and most research has been carried on it too. 2D BN lacks extensive investigation in its properties but the recent research has found many of their useful properties. BN nano-sheets are considered as one of the strongest electrically insulating materials. It can be made either mono or few atoms layer thick and unlike Graphene its strength does not decrease by increasing layers. The interlayer integrity of BN nano-sheets is significantly better than that of Graphene and make them a better and more suitable option for mechanical reinforcements. BN nanosheets are used as a substrate for Graphene because of similar structure and slight lattice mismatch between the two. BN nanosheets are excellent proton conductors and electrically insulator which makes them suitable candidate for applications in fuel cells. On one hand they are electrical insulator but on the other hand they have high thermal conductivity ranging from 100-270 W/ (m.K). BN nanosheets have the ability to be used as sensors and deep ultraviolet emitters and detectors. BN nanosheets are thermally stable. Mono atomic nanosheets is stable up to 800C in air. This makes them suitable for reinforcing ceramic and metal matrix composites. It is thermally and chemically inert and is, therefore, suitable for corrosion protection at high temperatures. Young's modulus of monolayer BN is reported to be  $0.865\pm 0.073$  TPa. Its fracture strength is  $70.5\pm 5.5$  GPa. Most importantly BN is white in color. As our project finds its application is bone and dental implants where white color is preferably required, we wish to use BN instead of Graphene in our project to retain the aesthetics and functionality. [6] [7] [8] [9]

## **Bone:**

The skeletal system is made up of various bones of different shapes and sizes. They protect the vital organs of our body and serve as levers for the muscle functions to make our body perform work. However, bones are not as simple as that. What looks to be a stationary part in the human body is prone to a lot of biochemical reactions that are occurring on and within the surface of the bones.

### Structure:

Generally, the bone consists of two layers of varying composition and definition. There is the strong and robust outer layer which is also termed as the “compact” layer that is considerably hard and denser in comparison to its counterpart. Then there is the spongy and less denser layer termed as “trabecular” bone with both having their own specific roles to play. Bones possess a tensile strength of 150MPa, a strain to failure of around 2% and a fracture toughness of roughly  $4\text{MPa (m)}^{1/2}$ .



*Figure 2 - The Compact and Spongy layers*

### Functionality:

Bones are also involved in the creation of blood cells which happens inside the bone marrow which is present in the trabecular region. This process requires a specific environment and it is one that the bones provide. Along with that, bones also serve as a storage area of sorts with deposits within the bone matrix composed of various minerals that are involved in the body. [10]

There are different cells performing different functions on the bone. These are termed as osteoclasts, osteoblasts and osteocytes. [11] [12] Together, they work on the degeneration and regeneration of the calcium on the bone and regulate it as necessary.



To specify each in detail:

**Osteoblasts:** These cells are responsible for synthesizing the new bone matrix.

**Osteocytes:** These cells are osteoblasts that become incorporated within the newly formed osteoid, which eventually becomes calcified bone.

**Osteoclasts:** Osteoclasts function in the digestion of mineralized tissue and are mostly found on the bone surface at sites of active bone resorption.

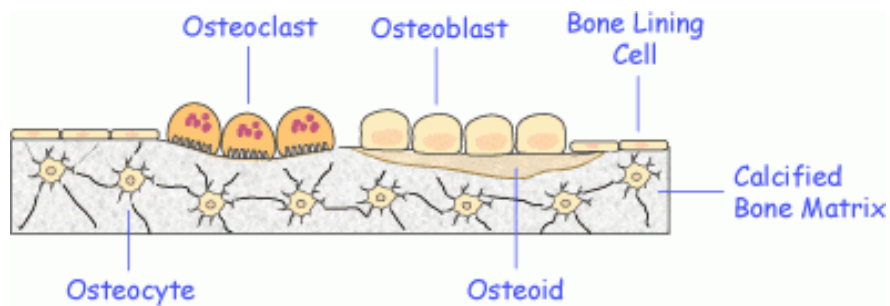


Figure 3 - Graphical Representation of the cells

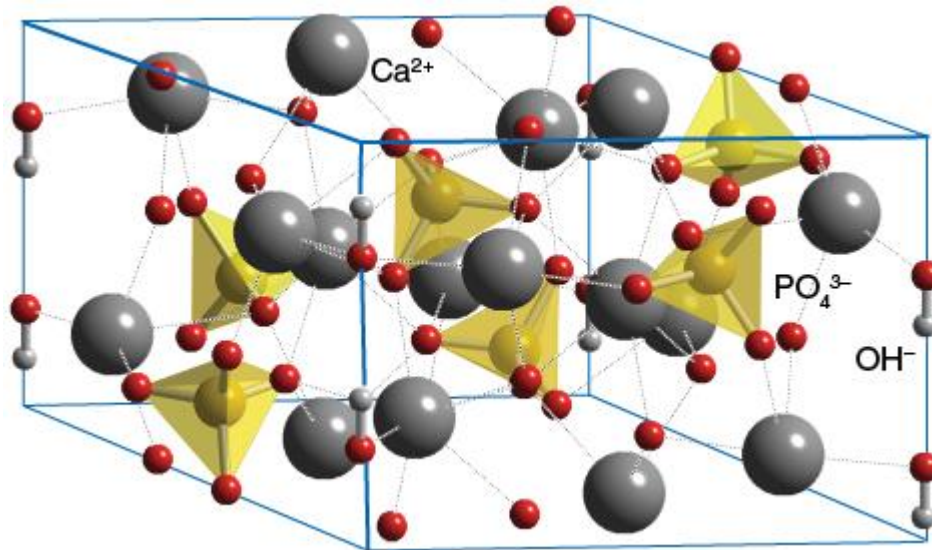
The calcium in the bones is constantly being varied due to the action of the thyroid and the parathyroid glands. [13] [14]

Whenever there is a deficit of calcium in the blood, the parathyroid gland produces parathyroid hormones which instruct the **osteoclast** cells on the bones to digest the mineral matrix which is present on the bone and works towards inhibiting the activity of the osteoblast cells which synthesize the new bone. This causes the calcium from the bone to be transferred into the blood stream and fulfil the calcium requirement. As a counter to the destroyed bone, new bone has to be created in its place. This is done by the thyroid gland promoting the production of **calcitonin** in the bloodstream by using the vitamin D present in the blood and it provokes the **osteoblasts** and **osteocytes** to produce the new bone by incorporating the calcium in calcitonin into the matrix of the osteoid and resulting

the formation of the new bone. Sometimes in the presence of excess calcium, a stronger layer of calcium on top of the bone is produced, thereby further reinforcing it.

### Hydroxyapatite (HA):

Hydroxyapatite is a bone mineral of dipyramidal structure that is found in abundance in the composition of bone. The chemical formula for HA is  $\text{Ca}_5(\text{PO}_4)_3(\text{OH})$  but is usually denoted as  $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$  to denote that the crystal unit cell comprises two entities.



*Figure 4 – Depiction of a Hydroxyapatite Lattice*

A modified form of HydroxyApatite makes up for 50% of the volume and 70% of the weight of a human bone. However, for synthetic replacement and biocompatibility, the regular form of HA is used when it comes to dental and orthopedic necessities.

Synthetic HA has the ability to attach itself to human bones and form a protective layer on top of them which can serve to reinforce and further enhance a damaged/degraded bone as well as tooth enamel which is why it is used in the orthopedic and orthodontic applications.

For the purpose of this thesis, the HA produced in the lab has been characterized as pure HA with minimal to no impurities present. HA alone however, is a precursor to of the

actual material that we aim to produce which will be synthesized by reacting HA with Boron Nitride. This is predicted to give it enhanced flexural strength and load bearing capacity whilst maintaining the biocompatibility and inducing bioactivity within it. [15]

### **Microwave Refluxing Method:**

Where Conventional heating usually involves the use of a furnace or oil bath, where the heating effect is provided by heat being generated on the walls of the container by means of either convection or conduction. Following the conventional method causes the core of the sample to take much longer to achieve the target temperature.

This is where microwave refluxing comes into play. The importance of microwave heating is that microwaves act as an internal heat source. This is caused by Microwaves being able to heat the target compounds without requiring the entire furnace to be heated to a specific temperature first. The microwaves directly provide energy to the molecules present throughout the material body and this results in faster heating and a reduction both time and energy being utilized.

However, due to the design and uneven absorption of the microwaves by the body of the material being subjected to its effects. It is commonly observed that these bodies are usually non-uniformly heated i.e. some areas of the body are more heated than the others. Thus giving rise to the phenomena of superheating.

Different compounds convert microwave radiation to heat by a different coefficient. Thus, causing some parts of a material to be heated faster than others and resulting in the above mentioned non-uniformity. However, the benefits over conventional means are considerable:

- reaction rate acceleration
- milder reaction conditions
- higher chemical yield
- lower energy usage

Thus, microwave heating method was also tested during the refluxing stage of our experiment as discussed later in the thesis. [16] [17]

# Chapter 3

## Experimental Techniques

The experimental procedure comprises of the synthesis of hydroxyapatite, the exfoliation of boron nitride nanosheets from bulk boron nitride, the formation of the composite of hydroxyapatite reinforced with boron nitride nanosheets, the coating of the nanocomposite powder on a metal substrate and Vickers hardness testing.

### Synthesis of hydroxyapatite:

Hydroxyapatite powder was synthesized via wet precipitation method using egg shells as a calcium source. The egg shells were collected and their inner membrane was removed as the first step. Next, they were washed with water to remove dust or other contaminants and then they were dried and ground to a powder from via means of crushing in a mortar and pestle. The egg shell powder was heat treated to 250<sup>0</sup>C for one hour and then at 1000<sup>0</sup>C for three hours in an oven. This resulted in a pure form of calcium oxide powder. [18] [19]

0.5 molar solution of calcium oxide was prepared whose initial pH was in the range of 13-14 (alkaline). To this, 0.3 molar solution of phosphoric acid was added drop wise at the rate of 30-40 drops per minute. A few mL of ammonia solution were also added to lower the pH of solution.



The resultant solution was separated in two halves. One half was refluxed for 3 hours using conventional heating method in a heating mantle where it was followed by 20 hours of stirring. The other half was refluxed for 5 minutes using microwaves in a microwave oven in pulse mode i.e. alternating between on and off modes for a specific period of time for each mode. The resultant precipitates of both solutions were separately filtered. The filtrates were then washed 3 times with distilled water using a centrifuge to bring their pH

near to 7. The acquired precipitates were then dried in a hot air oven at 80°C for 12-15 hours resulting in hydroxyapatite powder. The powder was again heat treated at 900°C for one hour and XRD of this powder was performed to confirm its phase purity. This method is a green chemistry route for the synthesis of hydroxyapatite powder. Both normal refluxing and microwave refluxing were done in order to compare any differences in the structural morphology and in the phase purity of the HA powder produced by either method.

### **Exfoliation of Boron Nitride nanosheets:**

A few grams of 3D boron nitride powder were dissolved in N-Methyl-2-pyrrolidone and the solution was sonicated in a probe sonicator for 48 hours. Then the solution was centrifuged and particles were collected separately at 3 different RPM ranges:

- 500 RPM
- 1000 RPM
- 1500 RPM

The particles were then filtered resulting in boron nitride nanosheets of different particle sizes. The objective of this step was to form the nanocomposite with all the three different particle sized nanosheets to check if any of them gave a significant superiority in properties over the others.

### **Formation of nanocomposite:**

The nanocomposite was formed via in-situ precipitation of HA with BN nanosheets. BN nanosheets were dispersed in iso-propyl alcohol using a bath sonicator. This dispersion was then added in the calcium oxide solution while the phosphoric acid solution was being added drop wise into the calcium oxide solution. The rest of the procedure was the same as that of the synthesis of simple HA. It resulted in a HA-BN composite powder with HA particles attaching themselves onto the BN nanosheets. This new powder was then heat treated in a vacuum using a tube furnace to remove the impurities. It was heat treated in vacuum instead of air to prevent the degradation of BN.

Finally, two composite powders were synthesized;

- One using the BN nanosheets formed at 1500 RPM
- Second with the BN nanosheets formed at 1000 RPM

### **Coating on the metal substrate:**

The next step was to find a means to deposit the formed composite powder onto a metal substrate that can be inserted into the human body and function as intended with the added biocompatibility and bioactivity of the synthesized composite powder.

To do so, 2 grams HA powder was dissolved in 100mL methanol via stirring. The metal substrate of stainless steel grade – 316L was placed on a hot plate. The solution was then sprayed onto the metal substrate using a spray gun from a distance of 25-30 cm. This resulted in a fine even coating on the surface of the metal substrate and the heated metal resulted in a proper binding between the interfaces due to the coating somewhat diffusing onto the surface of the metal. [20]

# Chapter 4

## Characterization Techniques

### Introduction

Different characterization tools were used for the characterization and testing of the composite and pure samples. To study the surface morphology of the powders used, the Scanning Electron Microscopy (SEM) was used in SCME. To identify and confirm the microstructure and the phase purity of the samples, X-Ray Diffraction (XRD) technique was used which was also available in SCME. It also identifies the crystallographic structure and lattice parameter of different phases present. To identify the different functional groups and bonds, we used the Fourier transformation infrared spectroscopy (FTIR) technique. To get a proper image of the surface topography of the sample, Atomic Force Microscopy (AFM) was used and finally, to test the mechanical properties, mainly, the overall flexural strength and hardness, Vickers hardness testing was used.

### X- Ray Diffraction technique:

X-Ray Diffraction is a non-destructive analytical technique used to identify the crystal structure of materials or phases present.

As the properties of materials are linked back to the atomic arrangement in crystals therefore the study of crystallographic structures and lattice parameter is of primary importance. For this XRD is used. Crystalline structure comprises of planes of atoms which reflect the incident X-Rays that fall on it at a particular angle, this phenomena is used to detect the d-spacing, planes and the structure of a material.

When X-rays generated by Copper in cathode ray tube are filtered and incident on the sample, their interaction produces constructive interference. Those waves which when satisfy the Braggs Law, are detected, processed and analyzed.

Bragg's Law:

$$n\lambda = 2d \sin \theta$$

Where:

- n is the order
- $\lambda$  is wavelength
- d is the inter planner spacing
- $\theta$  is the angle of incident beam

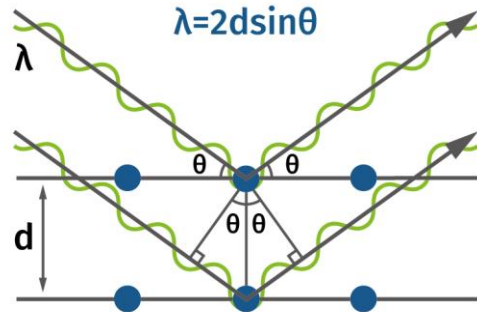


Figure 5 - Bragg's Law

This law relates wavelength of electromagnetic radiations to the lattice spacing in crystals and diffraction angle.

The sample is scanned in the range of  $2\theta$  so that all possible orientations and diffraction directions are attained. The machine measures angle  $2\theta$  by default as shown in the figure:

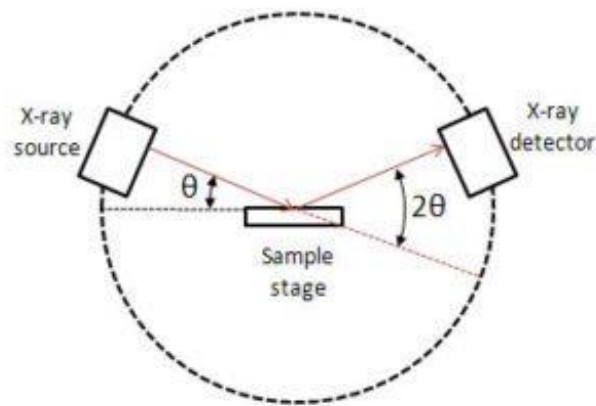


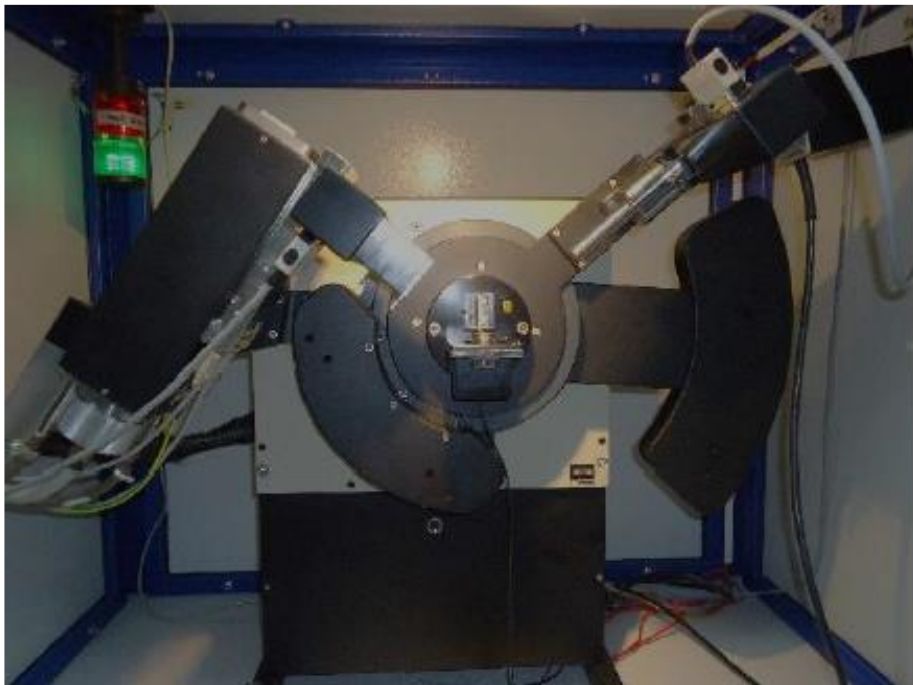
Figure 6 -  $2\theta$  Geometry

Diffraction peaks can be converted to d-spacing, this allows identification of the crystal structure and thus the materials because each crystal has its own unique d-spacing. This d-spacing is then compared with standard reference patterns available as cards in the reference library.



Limitations of XRD:

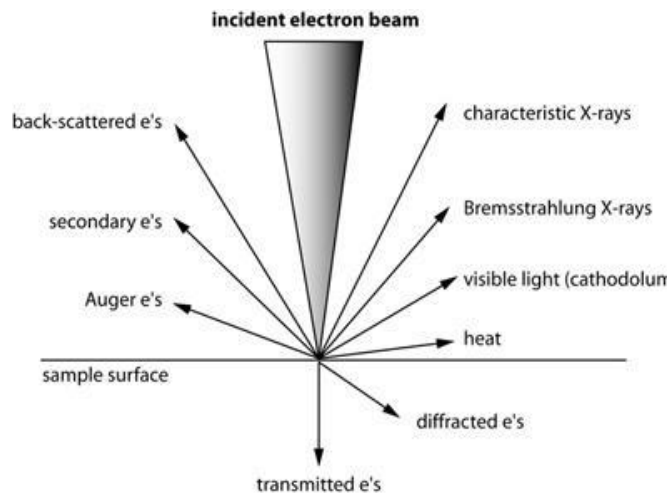
- If a materials to be identified is completely unknown it should be pure homogenous and single phase material to be properly detected and its peak identified.
- If less than 2% of a phase is present, it is not identified by XRD
- Sample preparation induce stresses in the materials thus changes in the d-spacing is observed, therefore resulting in shifted peaks



*Figure 7 - XRD Machine*

## Scanning Electron Microscopy

Scanning electron microscopy (SEM) is used to study the morphology and phases present in the material. SEM scans a focused electron beam over a surface to make an image. When electron beam is focused on a sample surface electrons interact with the sample therefore various signals are produced that can be used to retain information about the surface topography and the phase composition.



*Figure 8 - Scattering in SEM*

Some analysis that can be done using SEM include:

- Identification of materials
- Compositional analysis
- Topographical features, Morphology
- Phase distribution and crystal orientation
- Presence and location of defects. Failure analysis
- Elemental composition of micro-volumes with Energy Dispersive Spectroscopy (EDS)

The working of scanning electron microscope firstly involves sample preparation. The samples are first attached over a holder using double sided carbon tape. Thereafter, the samples are gold sputtered to increase the conductivity of electron beam through the sample.

Scanning electron microscopy is conducted by bringing a sample in contact to an electron beam. A powered electron gun generates a beam of electron with specific energy. Magnetic condensers are used to focus the beam and a specified rectangular area of specimen surface is focused. Interaction of the electron beam with material generated different signals which depends on the sample, i.e. backscattered electrons, auger electrons, photons, secondary electrons, etc. Specific special detectors are used to detect different types of signals being produced.

Signals are amplified to produce a magnified grey-scale image of the sample with extremely high resolutions in nanometer range. Magnification of scanning electron microscope is independent of the lens and is defined as the ratio of length of scan line on monitor and length of scan line on specimen. Thus the magnification is adjusted by changing the size of area of the specimen being scanned, i.e. the smaller the size higher would be the magnification.

Amount of signals detected from the surface vary due to difference in electronic densities of elements being used. The higher the atomic number of the element being scanned the more electrons are present and thus the interaction is larger therefore the elements with higher atomic number appear brighter than the lower atomic number elements.

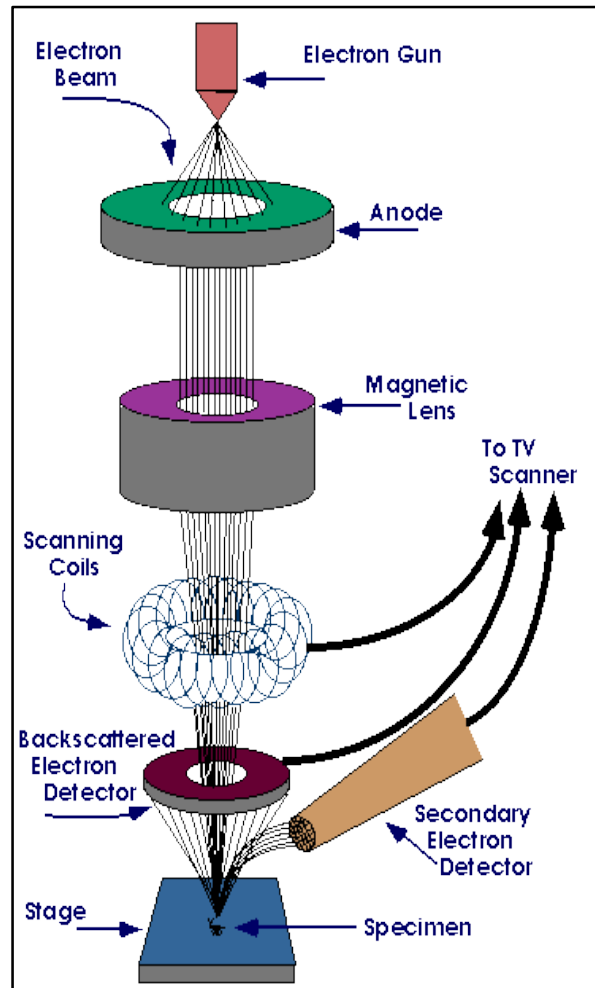
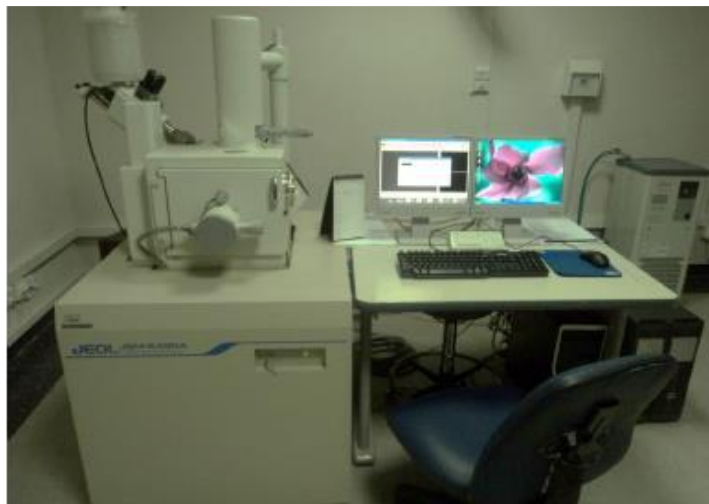


Figure 9 - Graphical representation of a SEM machine

SEM is performed on JEOL scanning electron microscope (JSM 6490LA) available in SCME. 5-20 kV operating voltage is used with spot size in the range of 30 to 55 and around 10mm of working distance is maintained.



*Figure 10 - SEM machine*

### **Fourier Transformation Infrared Spectroscopy**

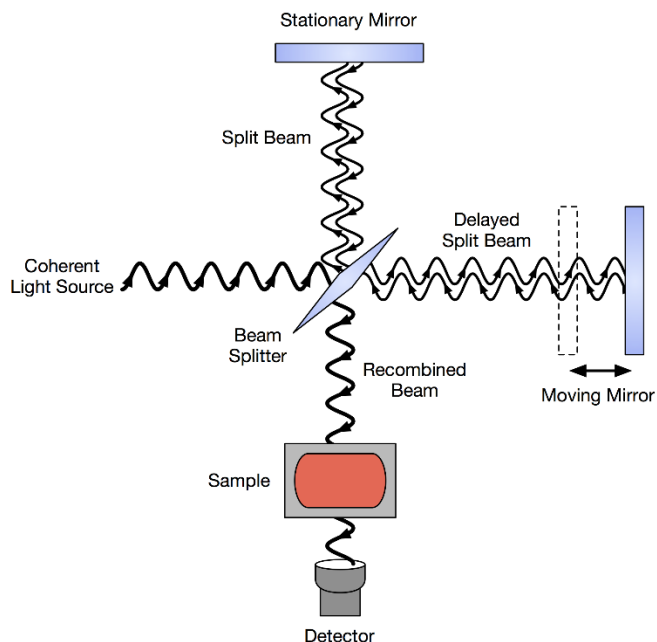
FTIR is an analytical technique used to study the atomic interactions between the molecules and between atoms within a molecule which are sensitive to Infra-red radiations.

Spectroscopy is referred to as the study of matter's interaction with electromagnetic radiation. All matter consists of molecules which have bonds that are continuously moving and vibrating. These bonds vibrate by stretching or bending motions. These bonds are sensitive to infrared radiation in the electromagnetic spectrum therefore each excited state is reached when the matter is exposed to specific frequency having the same energy as the difference between ground state and excited state of molecules/atoms.  $E = hv$ .

This phenomena is utilized to detect the functional groups or bonds present in the matter as excited state of particular atoms or molecules is achieved only when exposed to particular frequency. All these frequencies being absorbed lie in the IR region of the

electromagnetic spectrum which have a lower wavelength and higher energy than the visible light.

Sample preparation required for FTIR is simple but delicate. The powdered sample is mixed with Potassium Bromide (KBr) in a percentage of 0.2% - 1% and placed on the die which is pressed using hydraulic press at high pressure. This results in the formation of clear thicker pellet than films. These pellets are then used as a sample in FTIR machine.



*Figure 11 - Simplified representation of a FTIR Machine*

IR Spectrometer machine passes infrared radiation through a sample of an unknown compound which is mixed in KBr to form a pellet, the detectors are used to detect the transmitted rays which are then utilized to plot percent transmission of the radiation through sample versus the wavenumber of the radiation. Drop in transmission on y-axis represents the absorption of radiation of specific wavenumber. The energy of absorbed radiation is analyzed and compared from the literature to determine the material present. FTIR is a non-destructive technique and is a very quantitative way to identify the material present or the impurity type but qualitative in determining their concentrations.



*Figure 12 - FTIR machine*

## **Vickers Test**

Hardness is defined as a resistance of materials to indentation. That is, if we apply load to a material how hard it is to indent the materials determines the hardness of the materials. Usually hardness measurement through indentation is done by measuring the size or depth of indentation. Vickers test also known as micro-hardness test due the specifications it uses to measure hardness of specimen small in size and depth. Therefore sample preparation is also an important requirement for the Vickers test as the surface of the sample needs to be smooth and sample small enough in size to fit the apparatus, therefore sometimes mounting is also required to hold and deal with the sample easily.

Optical measurement is used to determine hardness by Vickers test. The procedure is as follows; square based diamond shaped indenter is loaded to the sample with small load of up to 30kg. This produces an indentation or mark on the specimen which then by using optical microscopy is measured in size as shown in the diagram. The dimensions are then substituted in the formula given to calculate the hardness value.

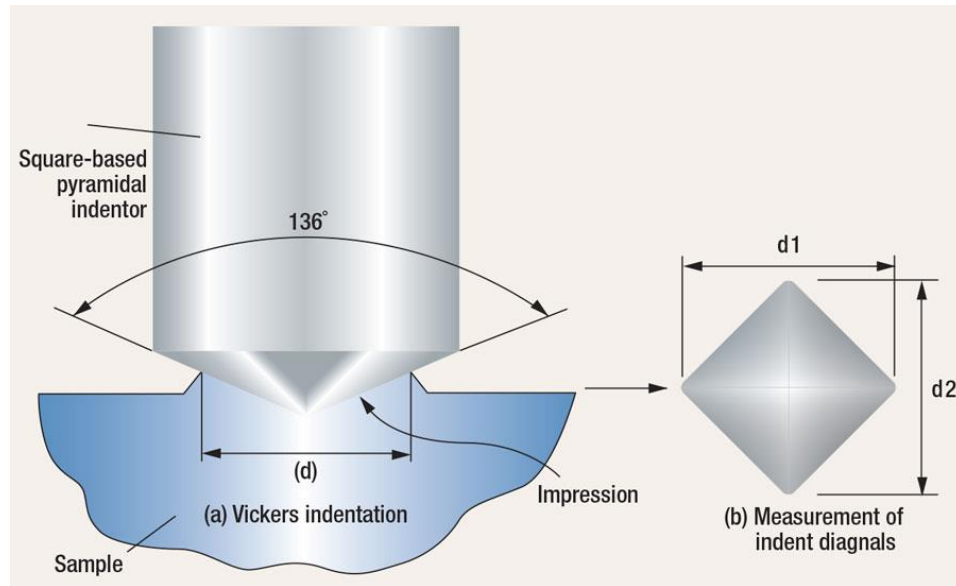


Figure 13 - Vickers Hardness indentation method

The formula to calculate the hardness value is:

$$DPH = \frac{2P \sin(\theta/2)}{L^2} = \frac{1.854P}{L^2}$$

- P is applied load in Kg
- L is average length of the two diagonal in millimeter
- $\theta$  is the angle between opposite faces of the diamond indenter that is 136°



# Chapter 5

## Results and Discussion

### XRD

Pure HA:

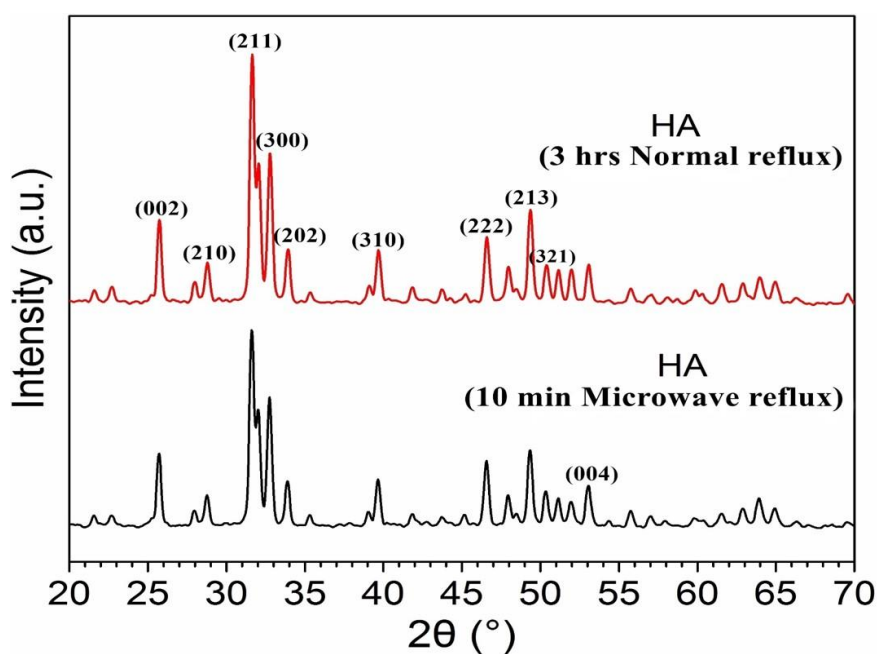


Figure 14 - XRD comparison of normal and microwave refluxed HA

This is the XRD graph observed for HA powder synthesized by two different refluxing methods. As it is visible both the graphs are finger print match of each other and no significant difference was observed in them. The graph is identical to the standard XRD graph of HA confirming that the HA synthesized was phase pure and no impurities were present in it. All the characteristic peaks for HA were observed. The crystallite size was calculated to be ~30nm using Debye Sherrer formula.

►  $d = 0.94\lambda/\beta\cos\theta$

BN nanosheets:

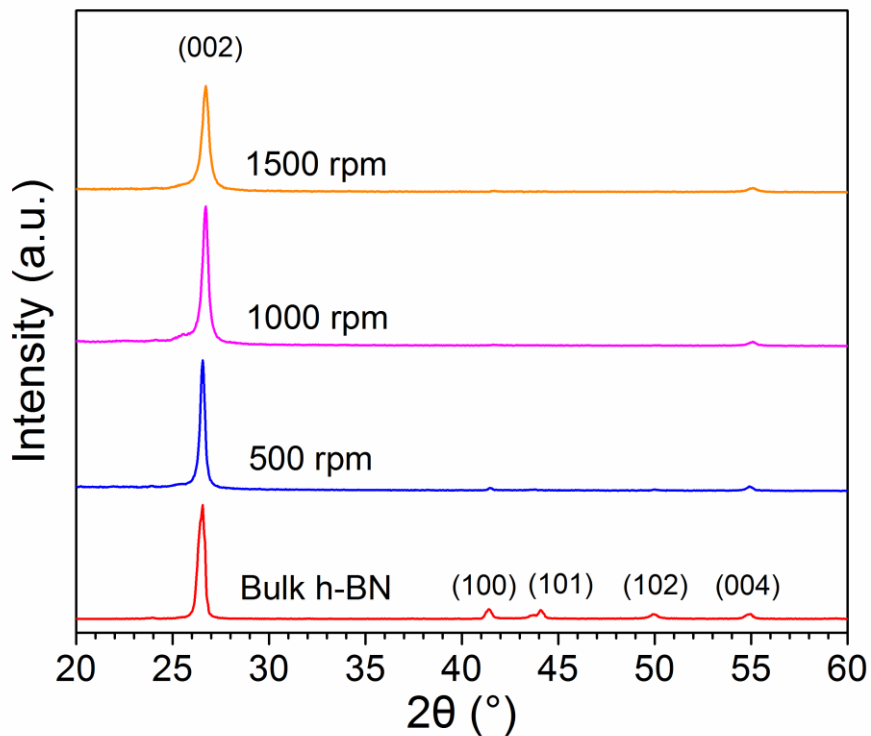
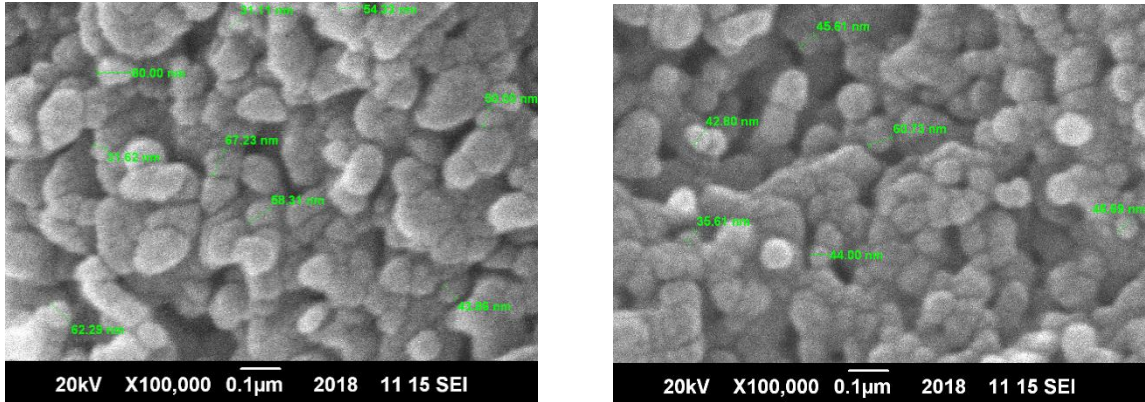


Figure 15 - XRD Comparison of bulk (3D) BN with 2D BN nanosheets formed at different centrifuge RPM's

XRD of BN nanosheets in comparison with bulk BN confirmed the phase purity of nanosheets. The characteristic (002) peak for 2D BN was obtained in all nanosheets while the other peaks of bulk BN were eliminated confirming the 2D structure and purity of nanosheets.

## SEM:

### Pure HA:

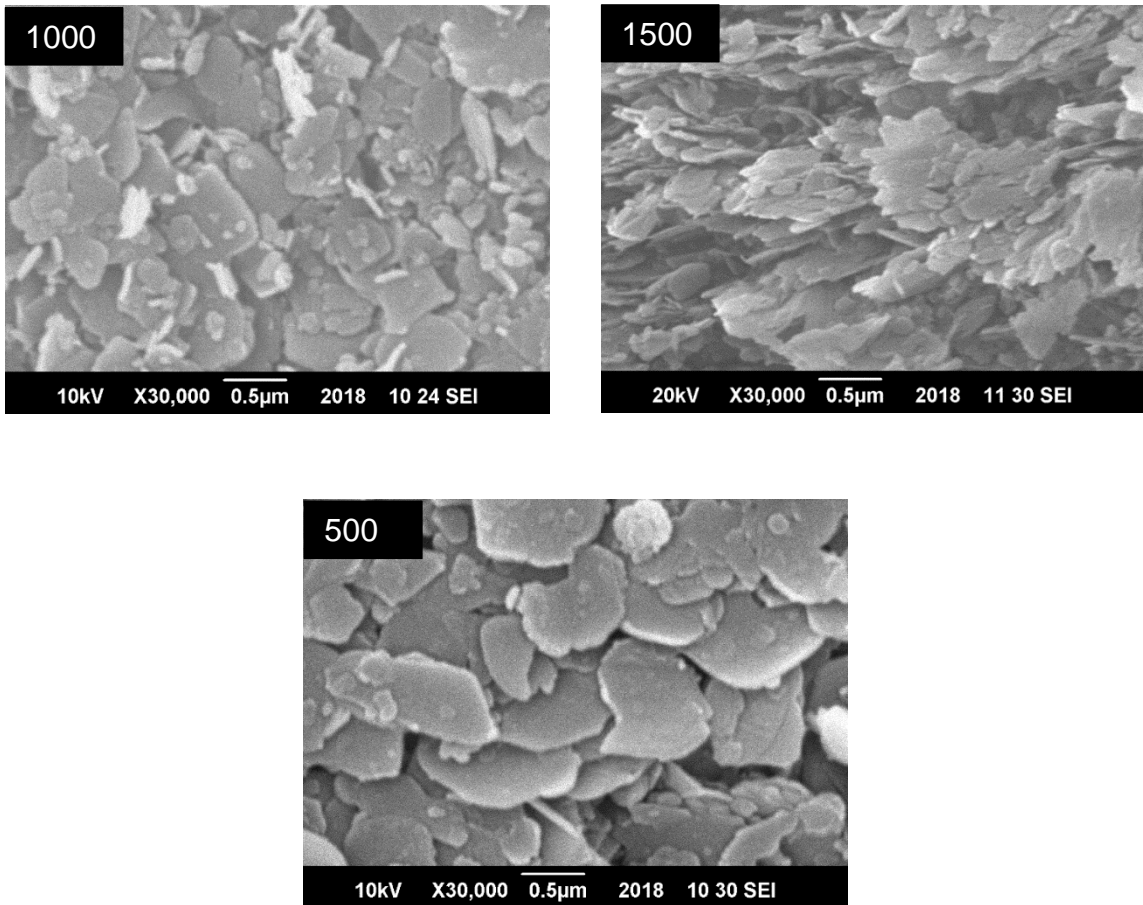


*Figure 16 - Microwave Refluxed HA (left) and Normal Refluxed HA (right)*

The SEM images show the morphology and topographical features of the HA powder synthesized by two different refluxing methods. Both the powders had identical morphology with nearly spherical particles. The average particle size for microwave refluxed HA was 75.5 nm while that for normal refluxed was 90.8 nm. No other significant difference was observed in the morphology of the two powders.

This proves that the microwave refluxing is a rapid and more economical production method for the synthesis of HA as it does not alter its properties when compared to HA powder synthesized by normal refluxing method which is more time consuming.

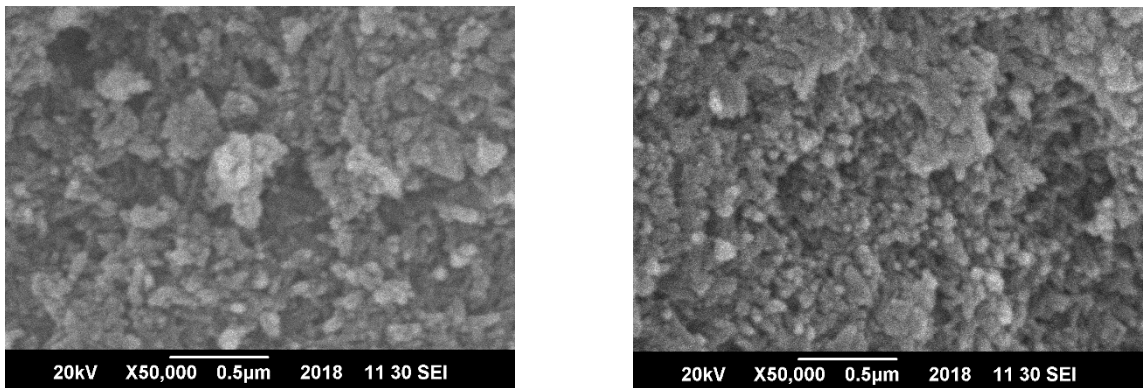
BN Nanosheets:



*Figure 17 - BN Nanosheets at different RPM ranges*

These SEM images show the morphology of BN nanosheets. Sheet like structure was obtained in all of them. Reduction in particle size as the rpm increases can be observed in the images with 500 rpm nanosheets having the largest particle size and 1500 rpm nanosheets having the smallest of them all.

HA-BN nanocomposite:



*Figure 18 - SEM of BN-HA composite powder with 1000RPM BN nanosheets (right) and 1500RPM BN nanosheets (left)*

The SEM images of nanocomposites confirm the incorporation of 2D BN into HA. BN nanosheets with HA particles attached to them can be observed in the images which confirm the achievement of heterogeneous nucleation which was the objective of in-situ synthesis.

## FTIR:

### HA:

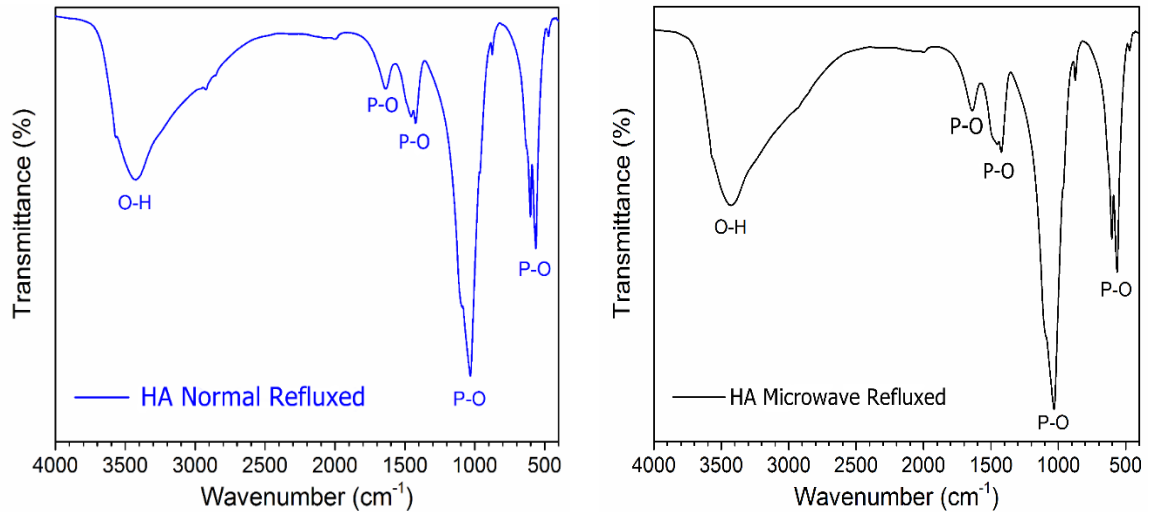
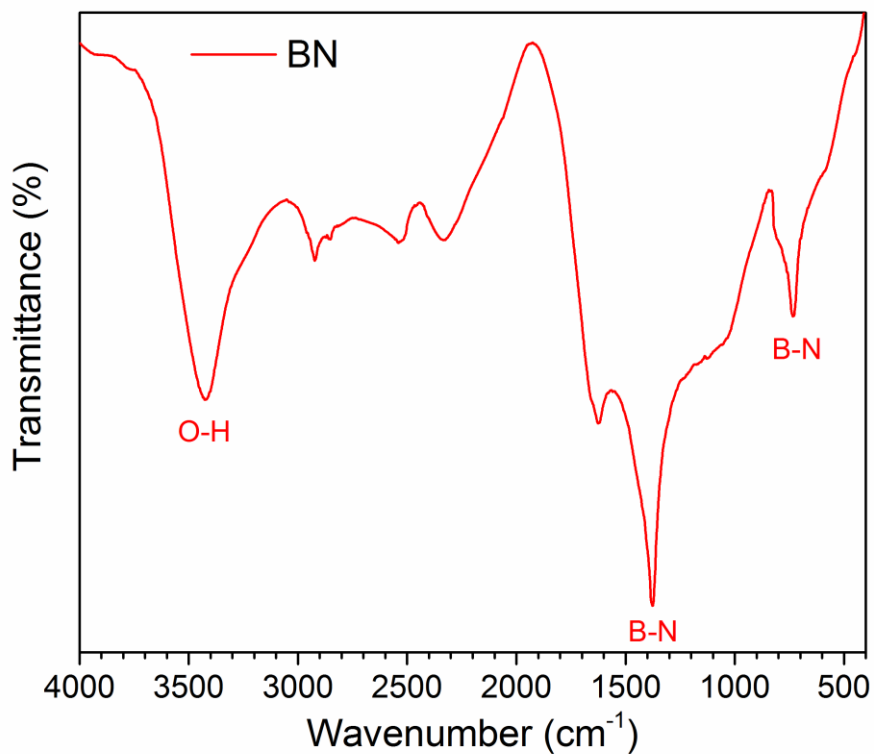


Figure 19 - Comparison between FTIR of Normal Refluxed HA and Microwave Refluxed HA

. The FTIR analysis of HA powder proves the presence of functional groups. Once again no difference in the two powders synthesized by different refluxing methods was observed. Both had identical peaks and they also matched with the standard FTIR graph for HA powder. All the characteristic peaks of O-H and P-O bond were observed and were labelled according to the findings of S. Koutsopoulos et al. [21]

These results again favored the microwave refluxing method over normal refluxing method as there was no difference in the FTIR graphs of both.

BN:



*Figure 20 - FTIR of Pure BN Nanosheets*

The FTIR graph of BN nanosheets confirmed the presence of O-H functional group and the characteristic bending and stretching modes of B-N bonds. The graph matches the standard FTIR graph for BN nanosheets provided in the reference literature.

HA-BN nanocomposite:

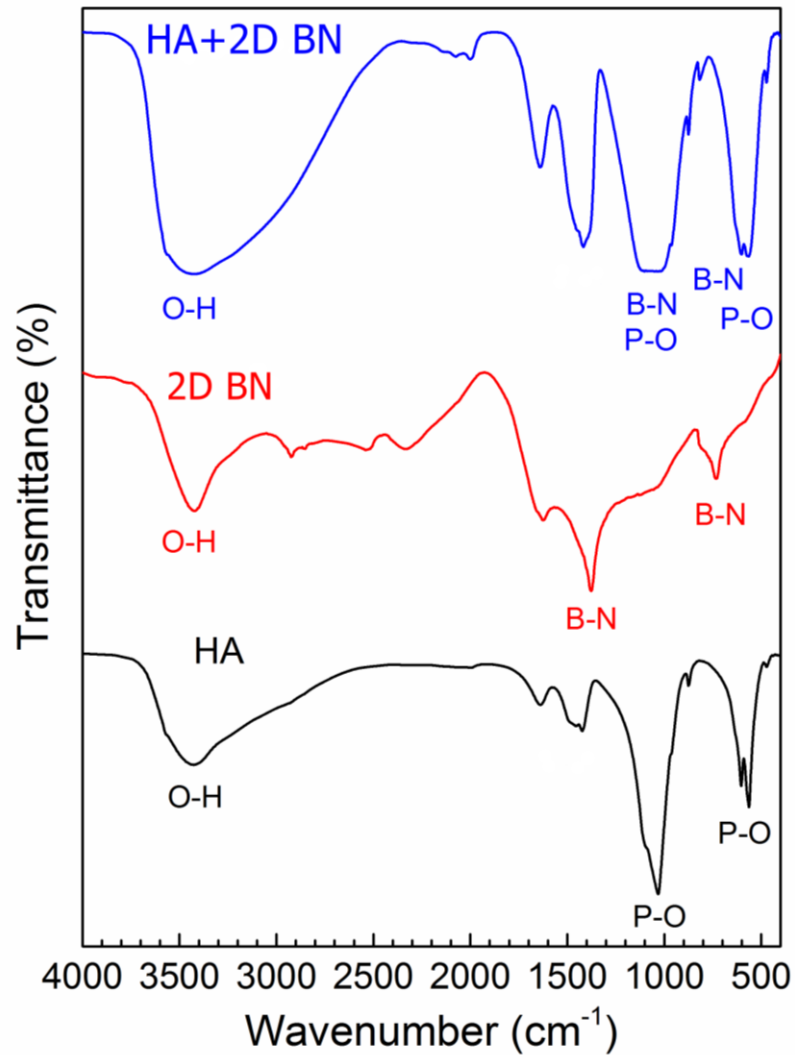


Figure 21 - FTIR Graph comparing pure HA, pure BN and the powdered HA-BN composite

The FTIR graph of nanocomposite shows a more stretched peak near the position of BN for BN nanosheets and PO for HA peaks which shows the presence of BN-PO interface in the nanocomposite. A broader peak shows the absorbance of infrared over a larger range of wave number which is due to the BN-PO bond interface.



### **Micro Vickers hardness testing:**

The hardness testing for our coated samples showed an increase in hardness. The average hardness value for sample coated with simple HA powder was found to be 220 Hv. The average hardness value for sample coated with nanocomposite coating was found to be 237 Hv.

## **Conclusion:**

From our experimentation and results, we have concluded that:

- Recycling of waste material i.e egg shells to synthesize HA powder was successful.
- Microwave refluxing proved to be the method of rapid and economical synthesis.
- The characterization techniques proved the incorporation of BN into HA powder confirming the successful synthesis of nanocomposite.
- Increase in hardness value proved the increase in mechanical property of the nanocomposite.

## **Future Direction:**

The increase in hardness value of nanocomposite coating suggests an improvement in its other mechanical properties as well. An investigation into other mechanical properties like toughness, fracture strength, tensile strength, impact strength, wear resistance etc will fully determine the potential of nanocomposite to be used in the field of biomaterials. Furthermore if the mechanical properties turn out to be promising, the in-vivo testing of composite either in the form of implant coating or scaffold will determine its biocompatibility and decide whether this material can be used on a commercial scale or not. If the mechanical properties are promising, this material may also find its use in dental implants as well.

## References

- [1] Ager, J. W., Balooch, G. and Ritchie, R. O. (2006) “Fracture, aging, and disease in bone,” *Journal of Materials Research*. Cambridge University Press, 21(8), pp. 1878–1892. doi: 10.1557/jmr.2006.0242.
- [2] Kasir, R., Vernekar, V. N. and Laurencin, C. T. (2017) “Inductive biomaterials for bone regeneration,” *Journal of Materials Research*. Cambridge University Press, 32(6), pp. 1047–1060. doi: 10.1557/jmr.2017.39.
- [3] Tuttle, B., Alhassan, S. and Pantelides, S. (2018). *Nanomaterials*. [online] Mdpi.com. Available at:  
[http://www.mdpi.com/journal/nanomaterials/special\\_issues/2D\\_nano\\_graphene](http://www.mdpi.com/journal/nanomaterials/special_issues/2D_nano_graphene)
- [4] Chopra, N., Luyken, R., Cherrey, K., Crespi, V., Cohen, M., Louie, S. and Zettl, A. (2018). Boron Nitride Nanotubes.
- [5] Song, L., Ci, L., Lu, H., Sorokin, P., Jin, C., Ni, J., Kvashnin, A., Kvashnin, D., Lou, J., Yakobson, B. and Ajayan, P. (2010). Large Scale Growth and Characterization of Atomic Hexagonal Boron Nitride Layers. *Nano Letters*, 10(8), pp.3209-3215.
- [6] Falin, A., Cai, Q., Santos, E., Scullion, D., Qian, D., Zhang, R., Yang, Z., Huang, S., Watanabe, K., Taniguchi, T., Barnett, M., Chen, Y., Ruoff, R. and Li, L. (2018). Mechanical properties of atomically thin boron nitride and the role of interlayer interactions.
- [7] [Online]. Available: <http://academic.pgcc.edu/~mhubble/a&p/205/notes/notes7.pdf>.
- [8] Zhi, C., Bando, Y., Tang, C., Kuwahara, H. and Golberg, D. (2009). Large-Scale Fabrication of Boron Nitride Nanosheets and Their Utilization in Polymeric Composites with Improved Thermal and Mechanical Properties. *Advanced Materials*, 21(28), pp.2889-2893.
- [9] Lin, Y., Williams, T., Xu, T., Cao, W., Elsayed-Ali, H. and Connell, J. (2011). Aqueous Dispersions of Few-Layered and Monolayered Hexagonal Boron Nitride Nanosheets from Sonication-Assisted Hydrolysis: Critical Role of Water. *The Journal of Physical Chemistry C*, 115(6), pp.2679-2685.

- [10] Huo, K. F., Hu, Z., Fu, J. J., Xu, H., Wang, X. Z., Chen, Y., Lü, Y. N., Liu, B. H. and Ding, J. (2003) "Chemical synthesis and characterization of boron/boron nitride core-shell nanostructures," *Journal of Materials Research*. Cambridge University Press, 18(7), pp. 1641–1645. doi: 10.1557/JMR.2003.0225.
- [11] Buckwalter JA, Cooper RR, "Bone Structure and Function"
- [12] "Normal Structure & Function of the Musculoskeletal System," Cleveland Clinic, [Online]. Available: <https://my.clevelandclinic.org/health/articles/normal-structure-and-function-of-the-musculoskeletal-system>.
- [13] 5 G. R. d. S. S. E. S.-C. J. S. a. P. S. C. Rinaldo Florencio-Silva, "Biology of Bone Tissue: Structure, Function, and Factors That Influence Bone Cells".
- [14] "Introduction to Bone Biology: All About our Bones," International Osteoporosis Foundation, [Online]. Available: <https://www.iofbonehealth.org/introduction-bone-biology-all-about-our-bones>.
- [15] M. Ishikawa, A. P. G. Salazar-Alvarez, A. P. Y. Oaki and P. H. Imai, "Synthesis of Hydroxyapatite/Nanocellulose Composites," 2014.
- [16] Suchanek, W. and Yoshimura, M. (1998) "Processing and properties of hydroxyapatite-based biomaterials for use as hard tissue replacement implants," *Journal of Materials Research*. Cambridge University Press, 13(1), pp. 94–117. doi: 10.1557/JMR.1998.0015.
- [17] Fang, Y., Agrawal, D. K., Roy, D. M. and Roy, R. (1994) "Microwave sintering of hydroxyapatite ceramics," *Journal of Materials Research*. Cambridge University Press, 9(1), pp. 180–187. doi: 10.1557/JMR.1994.0180.
- [18] Fang, Y., Agrawal, D. K., Roy, D. M. and Roy, R. (1992) "Fabrication of porous hydroxyapatite ceramics by microwave processing," *Journal of Materials Research*. Cambridge University Press, 7(2), pp. 490–494. doi: 10.1557/JMR.1992.0490.
- [19] T. M. G. a. W. E. B. E. C. Moreno, "Preparation and Solubility of Hydroxyapatite".

- [20] Chui, P. and Sun, K. (2014) “Thermal stability of a nanostructured layer on the surface of 316L stainless steel,” *Journal of Materials Research*. Cambridge University Press, 29(4), pp. 556–560. doi: 10.1557/jmr.2014.16.
- [21] S, K. (2018). Synthesis and characterization of hydroxyapatite crystals: a review study on the analytical methods. - PubMed - NCBI. [online] Ncbi.nlm.nih.gov. Available at:  
<https://www.ncbi.nlm.nih.gov/pubmed/12221709>.