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Effects of Increasing Chitosan Nanofibre Volume Fraction on the Mechanical Property of Hydroxyapatite

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Abstract This work attempted to synthesize chitosan (CH) nanofibre from crab shell and hydroxyapatite, HA, from limestone with the objective of studying the effects of increasing volume fraction of chitosan nanofibre on the mechanical properties of HA. Mechanical characterization of different fraction composite was carried out to study the effects of increasing volume fraction of chitosan nano fibre on the mechanical properties of HA. In addition, surface characterization of the composite was carried out using Fourier Transform Infrared Spectrometry, FT-IR. Results obtained indicated that the optimum mechanical properties were obtained at a volume fractions of 30: 70, CH: HA respectively; average compressive strength of 10.12 MPa; average tensile strength of 173.9 MPa; average hardness value of 420.80 HV; average fracture toughness of 14.72 MPa.m$^{1/2}$; average elastic modulus of 0.1583 GPa and average bending strength of 157.96 MPa were obtained for this optimum volume fractions. Increasing volume fractions of chitosan nanofibre was therefore found to result in decrease in compressive strength, hardness and elastic modulus of HA while its tensile strength, bending strength and fracture toughness increased. The FTIR revealed that possible interaction between the NH_2 group and the primary and secondary –OH group of CH with Ca^{2+} (metal coordination interaction) of HA might be responsible for the higher mechanical property of HA. In conclusion, it was found that increasing chitosan volume fraction in chitosan/HA composite results in increasing strength of hydroxyapatite, consequently enhancing its load bearing ability.

Keywords: *hydroxyapatite, chitosan, nanofibre, composite, volume fraction*

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1. Introduction

Various natural biocomposites exist. These are made from organic matrix and inorganic fraction and they fulfill the mechanical properties required in their functions as the skeleton, teeth or shells of organisms. For example, organic collagen combined with inorganic hydroxyapatite [HA: $Ca_{10}(PO_4)_6(OH)_2$] in bone and similarly chitin is associated with calcium carbonate in crab shell. These constitutions have very good mechanical properties that can withstand tremendous pressures [1].

Hydroxyapatite, which has been proved to have good properties of hardness, biocompatibility, osteoconductivity, a certain degree of bioactivity, and high resistance to moisture has been used in a variety of orthopedic, oral and maxillofacial applications [2]. It is available in dense blocks, porous blocks, and granules. However, each of these forms has its own drawbacks: Dense HA is difficult to machine without causing large-scale fracture, granules tend to migrate, and the macroporous material leaves a rugged surface finish. Generally, dense hydroxyapatite does not have the mechanical strength required to enable it succeed in long term load bearing applications; consequently, the need to improve its strength to enhance its load bearing application. One of the approaches to accomplish this is by incorporating chitosan nanofibre into its matrix. The purpose of creating HA-chitosan composite is to improve the mechanical property of hydroxyapatite.

Chitosan is a deacetylation product of chitin, which is a functional and basic polysaccharide composed of b-1, 4-linked glucosamine, that can be easily isolated from exoskeletons of shrimps and crabs [3]. Owing to its special properties such as nontoxicity, biodegradability, biocompatibility and antimicrobial activity, this polycationic biopolymer is receiving a great deal of attention for biosensing, medical and pharmaceutical applications [4,5,6]. Also, it is the most commonly used natural polymer in regenerative medicine and tissue engineering [7]. Chitosan nanofibres have also been widely accepted as biomedical scaffolding materials to restore, maintain or improve the functions of various tissues [8,9].

The substantial development of composite materials with CH mimics all the properties of bone. Calcium phosphate materials are osteoconductive and are able to mimic the inorganic portion of natural bone, while CH/HA composite materials show promise in mimicking the organic portion as well as the inorganic portion of natural bone [10]. Natural bone is composed of poorly crystalline nano-hydroxyapatite (n-HA) dispersed in collagen matrix. Hence, due to their analogy to bone matrix coupled with good biological and mechanical performances, development of nano HA –biopolymer based composites has received much more attention recently. Among various biopolymers, natural biopolymers find important applications in the field of orthopaedic and other biomedical fields, due to their excellent biocompatibility and biodegradability.

Various kinds of polymer/ceramic composite system such as HA/ collagen [11,12], HA/chitosan [13], HA/ collagen/poly (lacticacid) [14], HA/ alginate/collagen [15], HA/gelatin [16,17] were employed for preparing scaffolds for bone tissue engineering. Hydroxyapatite composites with CH matrices were extensively investigated in the last decade for structural purposes, specifically for orthopedics and maxillofacial surgery [18], either to fill bone defects, to increase the alveolar edge, middle ear implants, and fusion of spine vertebrae or to coat metallic prosthesis, due to proper filling-matrix integration [19]. Murugan *et al.*, used mixing approach to develop a simple procedure, by using the wet way, to generate nanostructured compound systems (nanocomposites) of HA and CH in two steps: HA precipitation in alkaline medium (pH 10.0 with NH_4OH) starting from $CaCl_2$ and $(NH_4)_2HPO_4$ as precursor salts and further mixing of nanometric HA with solutions at different CH concentrations in acetic acid at a fixed temperature [20]. Recently, a method of layered electrophoretic co-deposition of the CH-HA composite for protecting steel-made materials getting into contact with physiological solutions was developed [21] to modify the surface of shape memory materials made up of nickel-titanium alloys and protect them from corrosion. Additionally, some authors have studied the remarkable properties of coating technique by using biomaterials as Biovetir II, coated with nanostructured CH/HA composites to improve the implant interaction with cells and its fixation to the subcutaneous tissue [22] as well as for middle ear reconstruction [23].

Homogenous nano-HA/chitosan composite scaffolds with porous structure were prepared using a lyophilization method. Investigation of the microstructure, physical and chemical properties of the composite established that the porous nano-HA/chitosan composite scaffolds are promising materials in tissue engineering research [24]. In the same vein, hydroxyapatite-fucoidan (HApF) nanocomposite for bone tissue engineering was developed and characterized using different analytical techniques. It was observed that the prepared nanocomposite HApF have crystal size between 41 and 153 nm. The biochemical characterization affirmed that the prepared nanocomposite was non-toxic and that mineralization effect of HApF was two times higher than Hap, hence establishing that it could be used for bone tissue construct [25]. Solid three Dimensional (3D) Composite scaffolds for bone tissue engineering using the freeze-drying method was prepared. The Scaffolds were composed of chitosan, natural nano-hydroxyapatite (nHA) and fucoidan. The physiochemical constituents and the morphology of the scaffolds were investigated and chitosan-nHA-fucoidan composite was found to be a promising biomaterial for the design of scaffold for bone tissue regeneration [26].

In another investigation, homogeneous hydroxyapatite/ chitosan composite scaffolds were prepared via in-situ hydration method and characterized. The work established that homogeneous composite scaffold with better strength, bioactivity and biocompatibility can be prepared via in vitro hydration and this may serve as a good scaffold for bone tissue engineering [27]. Wassani and Whattanapong characterized porous scaffold from chitosan-gelatin/hydroxyapatite for bone grafting by using hydroxyapatite synthesized from mollusk shell via a wet chemical precipitation method and a commercialized chitosan. The result of the study established that the degradability and porosity of the scaffold increased with decreasing chitosan-gelatin and hydroxyapatite concentrations. However, swelling increased with increasing chitosan-gelatin but decreasing hydroxyapatite concentrations [28]. Mackay *et al.* worked on fabrication and mechanical properties of chitosan composite membrane containing hydroxyapatite particles and investigated the effects of hydroxyapatite content on the microstructure and mechanical properties of the composites. It was found that the Young's Modulus of the composites decreased while the failure strength and strain increased with the hydroxyapatite content [29].

Hydroxyapatite (HA) has been found as a suitable bone regeneration material. The brittleness of hydroxyapatite however limits its utilization in load bearing application. Hence, this study seeks to incorporate chitosan nanofibre into the matrix of HA with a view to influencing the strength and brittleness of the HA.

1.2. Description of Study Area

Lagos Lagoon is part of an intricate system of water ways made up of lagoons and creeks that are found along the coast line of Nigeria, Benin Republic, Togo and Cote d'Ivoire. It is connected to the Atlantic Ocean by Commodore Channel in Lagos. The lagoon is located between longitudes 3º 23' and 3° 40' E, and latitudes 6º 22' and 6º 38' N (Figure 1). It is the largest of the four lagoon systems, mainly of the Gulf of Guinea, covering an area of 257.49 km. The lagoon is shallow in most places, usually less than 1.5 m deep [30] and receives fresh water from important rivers like Yewa, Osun, Ona and Ogun [31]. The area surrounding the Lagos lagoon is probably the most urbanized and industrialized in Nigeria [32].

1.3. Description of Crab

Callinectes amnicola is a famous blue crab of the family Portunidae. It is an inshore and demersal estuarine crab species. It inhabits muddy bottoms in mangrove areas and River mouths [33]. The species is generally cherished source of protein and minerals in human diet and animal feeds [34,35]. Several physiologists have also used the blue crab as an experimental animal because of its ready availability, economic value, hardiness and complex life cycle [36]. Blue crab forms one of the most important sea food organisms and readily available in large quantity in West African markets. The distribution of the population is greatly affected by the salinity of the environment and climatic conditions.

2. Materials and Method

2.1. Raw Materials

The raw materials used in this research are**:** limestone collected from West African Portland Cement, Ewekoro, Ogun State, Nigeria and crab shells obtained from the Lagoon, Lagos State, South West Nigeria. The reagents used in this research include: 34.5 g of $(NH_4)H_2PO_4$ powder (99% MERCK), 317.97 g of Na_2CO_3 powder(99% ALDRICH), 2.05 kg of Sodium hydroxide pellet (99% ALDRICH), 100 ml of hydrogen peroxide, 25.21 g of oxalic acid powder (99% ALDRICH), Acetone (Sigma-Aldrich Laborchemickalien GMBH, Seelze, Germany), Hydrogen Chloride (HCl) and poly ethylene glycol (PEG).The equipment used in this work include: Jaw crusher, electronic oven, electronic balance, sieve, hot plate, magnetic stirrer, funnel, aluminium foil, spinneret, 5 ml syringe, pH meter, thermometer, mortar and pestle.

2.2. Method

The synthesis of chitosan from crab shell was carried out through deacetylation process. This was used to produce the chitosan nanofibre using electrospinning method. The hydroxyapatite was also synthesized from lime stone and the two were used to produce the chitosan nanofibre reinforced HA and characterized to study the effects of increasing volume fraction of chitosan nanofibre on some mechanical properties of HA.

2.2.1. Collection of Crab (Callinectes Amnicola)

The nocturnal and diurnal collections of crabs were conducted on callinectes amnicola in the Badagry Lagoon, Nigeria, between March to June 2016. Fishing gears such as non-return valve traps, gill and cast nets were implored in their collections, fishermen operating along the river were employed for the setting of gears and crabs collections. The crabs collected in the field were preserved immediately in 10% hydrogen peroxide solution in the laboratory prior to analysis. The sexes were identified by making use of the species conspicuous external morphological features, male by a T-shaped abdomen and females with their triangular or rounded aprons [37, 38]. The shells of male sex of callinectes amnicola (bivalve parts) belonging to the age groups 1 to 3 years was used in this research.

2.2.2. Synthesis of Chitosan from Crab Shells

The chitosan was synthesized from Callinectes amnicola shell using chemical hydrolysis method which involved four main processes as shown in Figure 2. They are (i) demineralization; (ii) deproteinization; (iii) discoloration and (iv) deacetylation. To produce 1.0 kg of 70% deacetylated CH, 6.3 kg of HCl and 1.8 kg of NaOH are required [11,12].

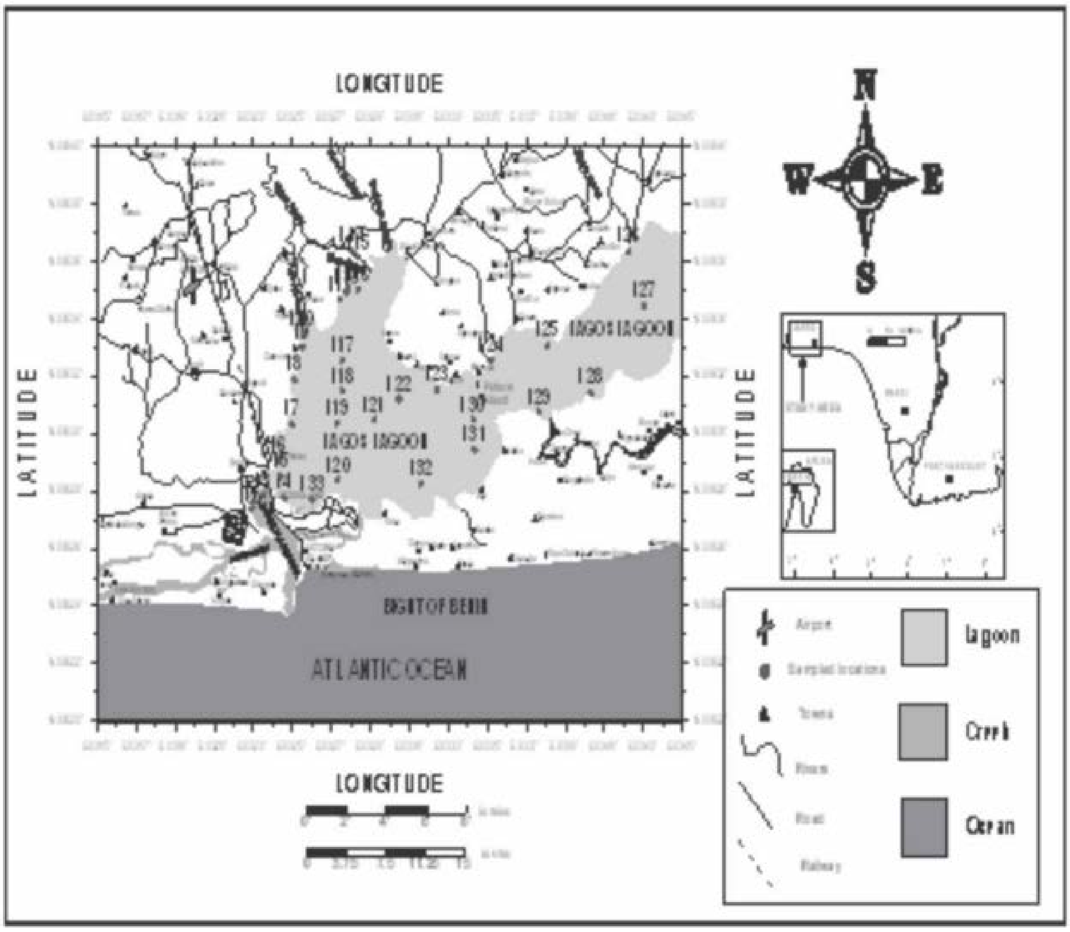

Figure 1. Map of Lagos Lagoon (Nigeria) Showing the Study Area

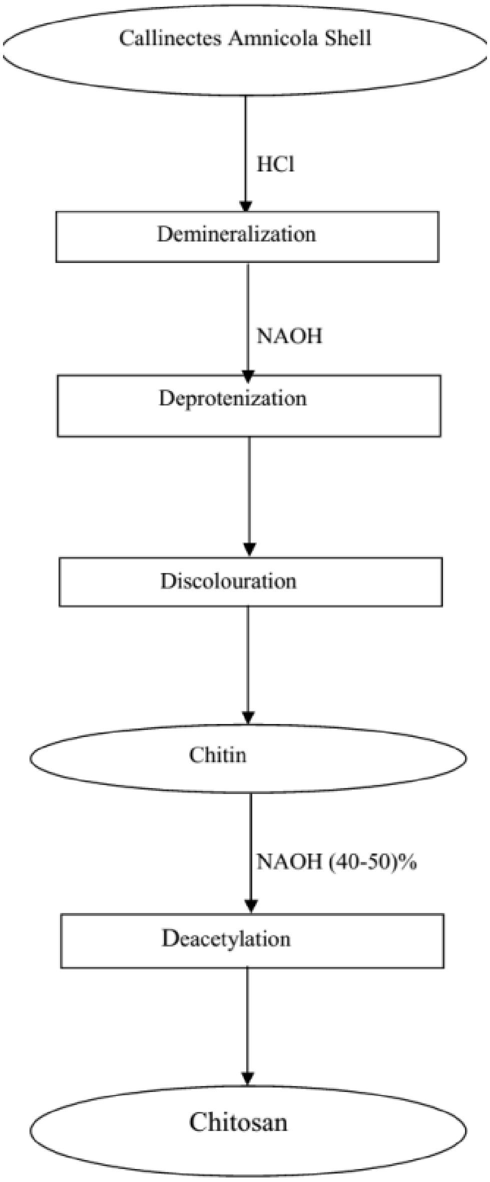

Figure 2. Flow chart showing the synthesis of chitosan from callinectes amnicola shell

2.2.3. Preparation of Chitosan Nanofibre

Chitosan was added to polyethylene glycol (PEG) as surfactant at ratio of 0.7 g: 0.3 g respectively. The mixture was dissolved in acetone at 10 ml/g and stirred at room temperature (37^{o}C) for 24 hrs using the magnetic stirrer. The homogenized solution was transferred into the spinneret through a 5ml syringe with 18 and 22 G blunt-end needle. The electrospinning was conducted in the Chemistry Laboratory of Centre for Energy Research and Development, Obafemi Awolowo University, Ile-Ife. The distance between the needle tip and aluminium foil collector was 12 cm and a voltage of 15-22 kv was supplied to the spinneret. The procedure was repeated and the resulting fibre was dried overnight to remove any solvent left on its surface and then collected from the collector.

2.2.4. Extraction of Hydroxyapatite from Limestone

Limestone was pulverized to obtain a fine powder of particle size less than 100 µm. 750 g of limestone powder was dissolved in 0.5 M of dilute HCl until the mixture ceased to produce carbon dioxide. Insoluble material such as silica was filtered out with filter paper. Oxalic acid was added to the residue to form Calcium oxalate as a solid precipitate. The precipitate was washed with deionized water and transfer into a beaker. The calcium oxalate was converted to calcium chloride solution through the addition of dilute HCl. Concentrated sodium carbonate was added to the calcium chloride solution to obtain Calcium carbonate precipitate. The precipitate was filtered off and the residue was washed with deionized water. The calcium carbonate was then dried in an oven at 105°C overnight and subjected to heating at100°C to produce calcium oxide. The Calcium oxide from limestone powder was converted to calcium hydroxide, $Ca(OH)_2$ by treating it with deionized water at 40°C for 1hr. About 0.3 M of ammonium fluoride solution and di-ammonium hydrogen phosphate solution were prepared separately. The two solutions were added to the aqueous suspension of the calcium hydroxide obtained and stirred vigorously. The solution was placed in an oven at 120°C for 1 hr after which the precipitate was filtered off with filter paper and the residue (HA) was washed with acetone and deionized water to separate it from impurity. The HA was oven-dried at 105°C for 24 hrs and the flakes produced were ground into powder of particle size of 100 µm.

2.2.5. Preparation of Hydroxyapatite/Chitosan Nanofibre Composite

The effects of increasing volume fraction of chitosan nanofibre on the mechanical properties of HA was studied by preparing composite made up of increasing volume fractions of CH (10 -90 Vol. %) Nanofibre in the HA matrix.

2.2.6. Mechanical Characterization of Composite

Different test specimens were prepared to analyze the mechanical properties of the different volume fractions of the composite in order to evaluate the effects of the chitosan nanofibre on the mechanical properties of hydroxyapatite and to determine the volume fraction with the optimum mechanical properties. The mechanical properties investigated include the compressive strength, tensile strength, hardness, fracture toughness, modulus of elasticity and bending strength. The average values of five (5) samples each of the properties investigated were determined, recorded and the values were plotted.

2.2.6.1. Compression Test

Different volume fractions of the composite were moulded with XQ -2B Inlay moulding machine into pellets of 30 mm diameter and 10 mm thickness using a uniaxial pressing machine at Materials Science and Engineering Laboratory, Obafemi Awolowo University (OAU), Ile-Ife, Nigeria, for the determination of compressive strength. Compressive stress was applied uniaxially to the pellets with a crosshead speed of 2 mm/minute at Material Testing Laboratory of Centre for Energy Research (CERD), OAU Ife, to determine the behavior of the composites under a compressive load and the stress versus strain values were plotted.

2.2.6.2. Tension Test

Tension test was carried out on the tensile specimen for the different CH/HA volume fraction composites using an instron universal testing machine, at a strain rate of 5 mm/minute. The test was carried out at Center for Energy Research and Development (CERD), OAU, Ile-Ife, Nigeria.

2.2.6.3. Hardness Test

The hardness of the different volume fractions of composite were analyzed with Vickers hardness test. The samples were placed on an anvil that has a screw threaded base in a universal testing machine. The anvil was turned raising it by the screw threads until it was closed to the point of indenter. With the start lever activated, the load was applied slowly to the indenter for 15 seconds. At the initiation of a macro crack, the experiment was stopped and the crack length was measured by means of digital vernier caliper. Measurements were taken across the diagonals to determine the area of indentation, after which the experiment was continued and the process was repeated for all the samples. The Vickers hardness (HV) was calculated with the help of Vickers hardness using equation 1

$$HV = 1.854\left(\frac{F}{C^2}\right) \tag{1}$$

Where:
HV = Vickers hardness value (HV)
F = Load applied (kg-f)
C = Diameter of indentation in (mm).

2.2.6.4. Fracture Toughness Test

The fracture toughness (K_{Ic}) of the different volume fractions of the composite was determined using the method of fracture by indentation, using equation 2 [39].

$$K_{IC} = 0.16(\frac{c}{a})^{-1.5}\left(Ha^{\frac{1}{2}}\right) \tag{2}$$

Where a = half length of the indentation diagonal, c = half length of the crack generated by indentation and H = hardness value.

2.2.6.5. Modulus of Elasticity

The modulus of elasticity (E) of the different volume fractions of the composite was evaluated using the slope of the tensile stress – strain curve.

2.2.6.6. Bending Strength

The bending strength of the different volume fractions of the composite was evaluated using equation 3 by [40].

$$BS = 15.1E^{0.85} \tag{3}$$

Where BS = Bending strength in MPa and E = elastic modulus in MPa.

2.2.7. The Fourier Transform Infrared Spectroscopy (FTIR) Analysis

The FTIR analysis was carried out in order to identify the main functional groups and the presence or absence of carbonate ions in the CH/HA composite. This was carried out using thermo Nicolet Magna 860 and continuum IR microscope. The composites were put in a vacuum oven at 50°C for 48 h before they were ground to a suitable size for IR analysis with spectrometer. The pellet for the FTIR measurement was prepared by mixing the sample (2mg) with 200 mg of IR-grade KBr. The absorption spectra were measured using spectrum 100 spectrometer at a wave number of 4000-400 cm^{-1} with a resolution of 4 cm^{-1} at CERD, OAU, Ile- Ife.

3. Results

The different volume fractions of the composites synthesized in the cause of this work are shown in Table 1, Table 2 indicates the hardness value of the CH/HA composite while Table 3 presents the peaks of infrared spectra attributed to HA/CH nanofibre composite. Result obtained from the compressive strength test for the different composite volume fraction is shown in Figure 3. Similarly, Figure 4 shows the tensile strength obtained for the different composite volume fraction and in the same vein, the Plot of fracture toughness, elastic modulus and bending strength against the different volume fractions are presented in Figure 5, Figure 6 and Figure 7 respectively. The Fourier Transform Infrared Spectroscope (FTIR) for the composite is shown in Figure 8.

Table 1. Different Volume Fractions of Chitosan/Hydroxyapatite Composite Samples

Sample	CH (Vol %)	HA (Vol %)
1	10	90
2	20	80
3	30	70
4	40	60
5	50	50
6	60	40
7	70	30
8	80	20
9	90	10

Table 2. Hardness Values of the CH/HA Composite

Composite Volume Fractions	I	II	III	IV	V	Average (HV)
10/90	320	325	303	317	328	318.60
20/80	368	357	350	368	358	360.20
30/70	422	418	401	433	418	420.80
40/60	388	392	381	397	379	387.40
50/50	403	415	393	420	410	408.20
60/40	418	427	411	405	423	416.80
70/30	343	338	345	350	347	344.60
80/20	371	384	395	388	373	382.20
90/10	275	255	272	240	286	265.60

Table 3. Peaks of Infrared Spectra Attributed to CH/HA Composite

Compound	Functional group	Infrared frequency (cm^{-1})
HA	Carbonate (CO_3^{-2})	1375
HA	PO_4^{-3} bend v_3	1027
HA	PO_4^{-3} bend v_4	532
HA	Structural (OH)	3425
Chitosan	Methylene ($-CH_2$)	2878
Chitosan	Amide I (C=0)	1662
Chitosan	Amine ($-NH_2$)	1582
Chitosan	Amide II (-NH)	1533

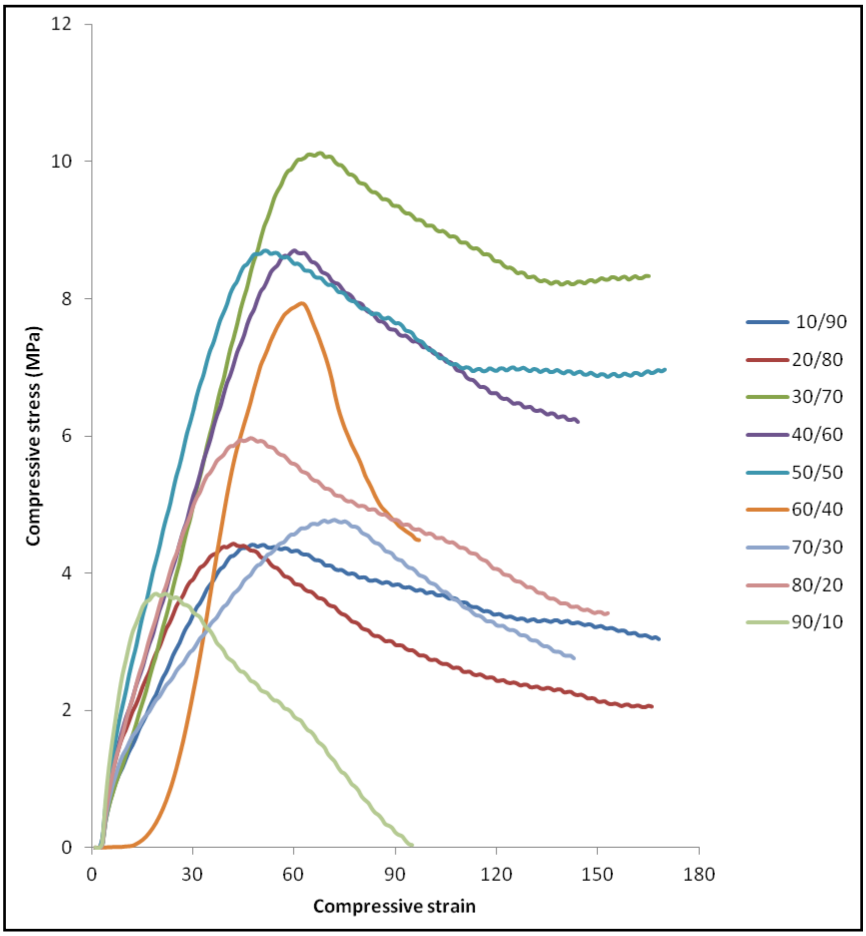

Figure 3. Compressive Strengths of CH/HA Composite

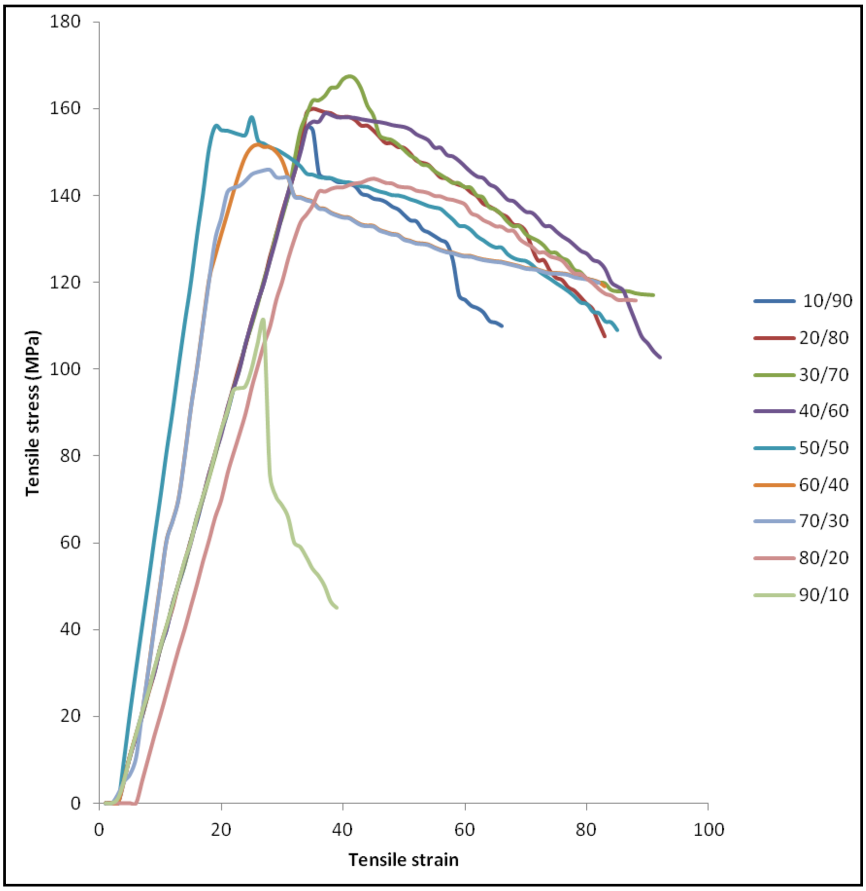

Figure 4. Tensile strengths of CH/HA composite

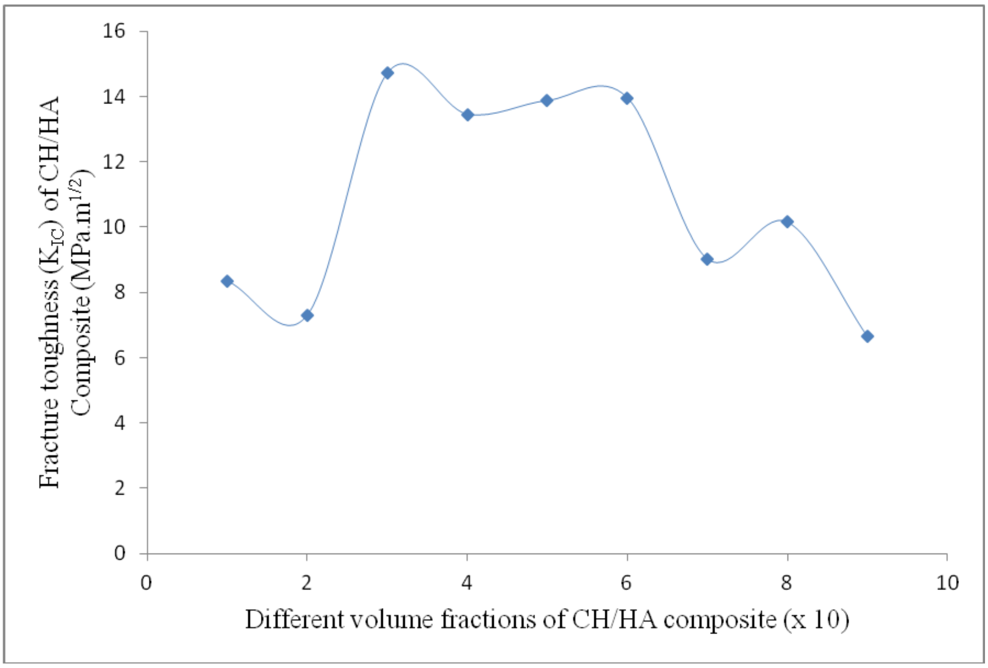

Figure 5. Fracture toughness characteristics of HA/CH nano composite

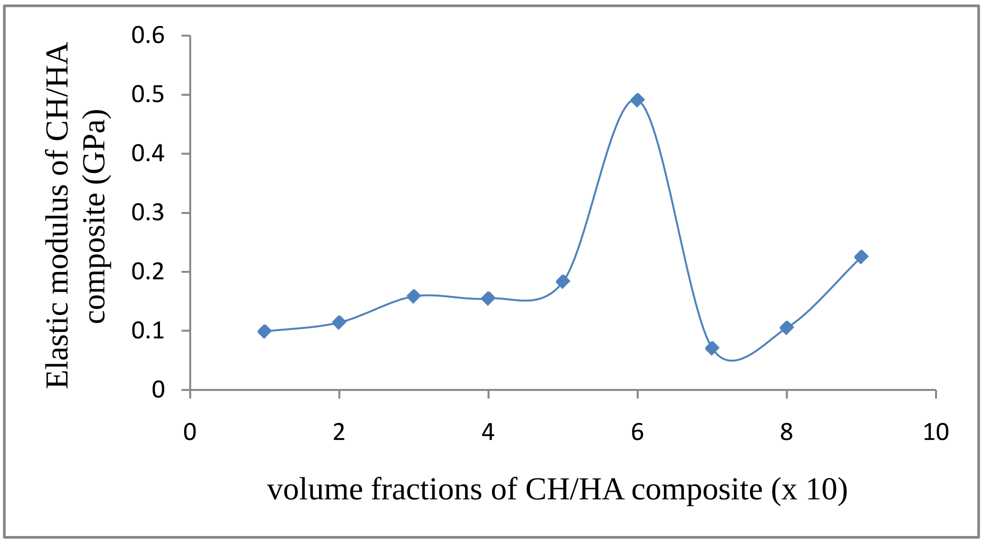

Figure 6. Elastic Modulus of CH/HA Composite

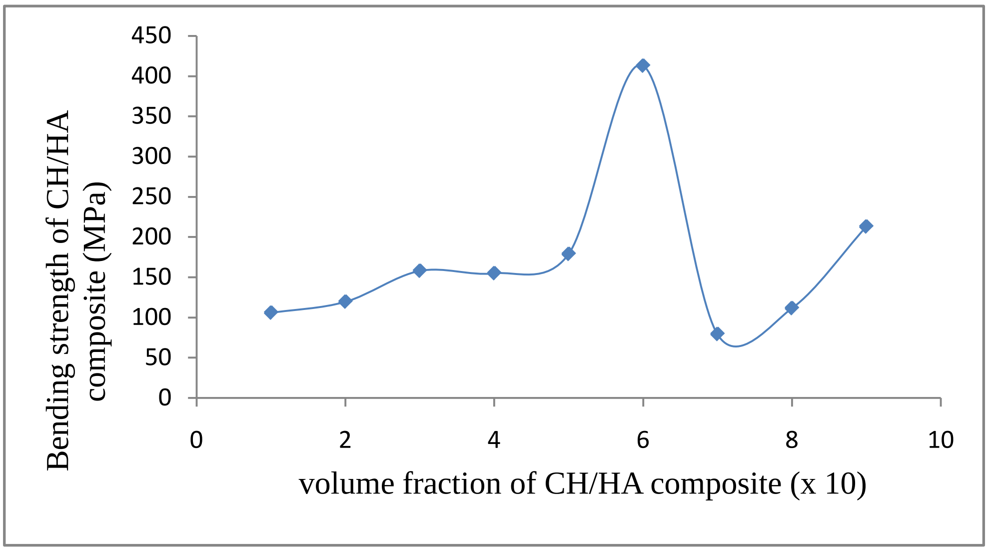

Figure 7. Bending Strengths of HA/CH nano Composite

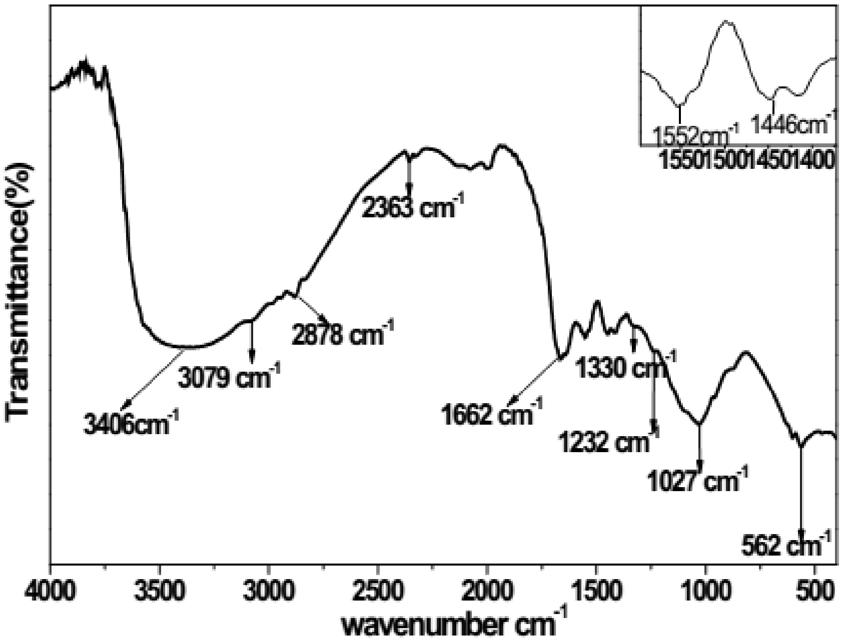

Figure 8. Fourier Transform Infrared Spectroscope (FTIR) of the CH/HA Composite

4. Discussion

4.1. Mechanical Properties

Hydroxyapatite, though a candidate material for bone regeneration is, however, brittle and therefore not suitable for load bearing application. The incorporation of nanofibre of chitosan into the HA matrix has been found to have pronounced effects on the mechanical properties of HA [41]. Increasing the volume fraction of chitosan from 10 - 90 % as shown in Table 1) resulted in a corresponding effect on mechanical properties of the composite (see Figure 3 to Figure 7).

4.1.1. Compressive Strength

Mechanical properties of HA play a significant role in its load bearing capability. The compressive strength of dense HA is within the range of 500 – 1000 MPa [42]-[43]. Increasing volume fraction of chitosan in HA had been reported to result in decreasing compressive strength [41,42,43,44]. Result obtained here is also observed to follow this trend as increasing volume fraction of CH was found to result in corresponding decrease in compressive strength. However, the composite with optimum volume fraction (30/70 – CH/HA), was observed to have the highest average compressive strength, 10.12 MPa, when compared to that of other volume fraction (Figure 3). This may be ascribed to the fact that increasing CH volume fraction may have affected the incorporation of the fibre into the matrix resulting in lower adhesion between the matrix and the reinforcement [44]. The compressive strength obtained is however found to be within the range of human cancellous bone, which is in the range of 2 – 12 MPa [45].

4.1.2. Tensile Strength

Increasing volume fraction of chitosan nanofibre has been found to increase the tensile strength of the composite (Figure 4). The tensile strength of dense HA was found to be within the range of 40 – 100 MPa [43]. As the volume fraction of CH was increased a corresponding increase in tensile strength was observed until an optimum composition was attained (Figure 4) and this behavior might be attributed to increase in interaction or contact between the reinforcement particles within the polymer matrix at high volume fractions [46]. After the optimum volume fraction of 30/70, a decreased in tensile strength was however observed. i.e. hydroxyapatite decreased the tensile strength of the composite with increased reinforcement as HA acts as ‘flaws’ in the polymer matrix as a result of poor interfacial bonding or decrease in the force of adhesion between the HA matrix and CH reinforcement fibre [47]. The optimum volume fraction 30/70 was found to have an average tensile strength of 173.9 MPa, which is in the range of the tensile strength of cortical bone, 50 – 151 MPa, [42,48,49]. Thus, the composite with the optimum volume fraction will be a good load bearing material.

4.1.3. Hardness

Hardness is one of the most important parameters of materials when considering the suitability of the materials for clinical use. In bone regeneration, it is desirable that the hardness of the biomaterials be comparable to that of bone. If the hardness of the implant is more than the bone then it penetrates into the bone [50]. The hardness of dense hydroxyapatite is between 500 to 800 HV [43]. The effect of increasing CH nanofibre in HA matrix was observed to result in a corresponding decrease in the hardness of the material (Table 2) but the optimum hardness value is observed at the composite volume fraction 30/70.

4.1.4. Fracture Toughness

It is reported that high fracture toughness material improved clinical performance and reliability compare to low fracture toughness [51]. According to [43], the fracture toughness of hydroxyapatite is approximately 1

MPa.m$^{1/2}$ which is very low when compared to the fracture toughness of human cortical bone which has value ranging from 2 – 12 MPa.m$^{1/2}$ [45]. Increasing CH volume fraction results in a corresponding increase in the fracture of the composite (Figure 5). The composite with the volume fraction 30/70 was observed to have the optimal fracture toughness of 14.72 MPa.m$^{1/2}$. This might be attributed to the chemical compatibility of the composite which results in high adhesion between the HA matrix and the CH nanofibre. This helps to obtain homogeneous microstructures with the formation of interfaces, which allow the activation of different mechanisms that enhance the transferred of loads efficiently and prevent cracking or fibre/matrix de-bonding when stressed. Thus composite with volume fraction 30/70(CH/HA), with high fracture toughness will enhance clinical performance and service reliability in load bearing application. Also, all types of bone experience the generation of micro-cracks (damage) before failure. Micro cracks start at yield and depending on the size, amount and interaction of such micro-craking, bone can be toughened to a greater or lesser degree to prolong its post yield deformation [52]. The load that creates the first micro crack is the maximum load because afterwards the material simply disintegrates more or less easily. Fracture toughness can be used for strength predictions. Failure is at the point where a major macro crack is initiated and the material strength is defined [53].

4.1.5. Elastic Modulus

The composite synthesized in this work can be a perfect match as a load bearing implant to prevent stress shielding since its modulus is strongly correlated with the modulus of human cancellous bone, which is in the range 0.010 – 2 GPa [54]. The effect of the chitosan nanofibre on the matrix of HA reduced the modulus of elasticity of the composite (Figure 6). It can be observed from Figure 6 that composite volume fraction 60/40 (CH/HA) has the optimum value of 0.4904 GPa while 0.1583 GPa value for the 30/70 volume fraction. Modulus of elasticity (E) is extremely important for biomedical implants. When an orthopaedic or dental implant is placed in the body to replace a bone or part of a bone, it is required to handle the loads in the same way as its surrounding bone. If the modulus of elasticity of the implant is higher than that of the bone being replaced, the implant will take over the load bearing, which results in stress shielding and eventually death of the surrounding tissues. Consequently, loosening of the implant and eventually failure may occur. When this happens, the patient will need a revision surgery to replace the implant [55]. The elastic modulus of hydroxyapatite according to [45] is 80 – 110 GPa and this is much higher than the modulus of bone and hence, cannot be used in load bearing application but the values of the modulus of the composite obtained are within the range of the modulus of human bone [54].

4.1.6. Bending Strength

Bending strength can be best explained as a function of yield strain. Yield strain is similar for all bone, although it declines slightly as elastic modulus increases. Nevertheless, the bending strength cannot be simply explained by assuming that the bone yield at some strain, which is a negative function of elastic modulus and that the bending moment at failure, is predicted by strain. When a sample is bent, one side is loaded in compression and the other in tension. Strain varies continuously, and in theory linearly, with distance from the neutral axis. If a material is homogeneous and behaves linearly elastically, the stress also varies linearly. The proportionality of stress and strain ceases. The sample may then undergo a greater bending moment, which might result in maximum bending stress. This may be spurious [56] because the stress at a particular strain is nearly proportional to elastic modulus. For a similar yield strain, a high elastic modulus will be associated with a high yield stress and therefore, a high bending moment when the sample yields. If the sample further shows a reasonable amount of post yield strain, then the bending moment will continue to increase for a while, and apparent bending strength will be higher. If the bone has a low modulus, it will yield at a rather low stress and even the large amount of post yield deformation characteristically shown by low modulus samples will not increase the apparent bending strength sufficiently to make up for the low stress at yield [56]. The bending strength of dense hydroxyapatite is 20 – 80 MPa [43] while that of human cortical bone is 50 – 150 MPa [40]. Since the bending strength of the composite volume fraction 30/70 is similar to that of human cortical bone, it may therefore be inferred that it will be a perfect bone substitute to prevent stress shielding. In Figure 7, the ultimate bending strength (412.99 MPa) was obtained at 60/40 (CH/HA) composite volume fraction, while the value at 30/70 (CH/HA) is 157.96 MPa. However, the bending strength of CH/HA 30/70 will be the most acceptable for bone substitute when compared with 60/40 CH/HA volume fraction since it is within the range of human cortical bone [40]. The factors responsible for the mechanical behavior of the composite can be attributed to particle size and particle size distribution of chitosan, interfacial interactions between the chitosan nanofibre and HA; and good distribution of nanofibre in the matrix of HA.

4.2. Fourier Transform Infrared Spectroscope (FTIR)

Figure 8 presents the FT-IR spectrum of CH/HA composite nanofibre. The characteristic bands for different functional groups present in the composite are presented in Table 3. Two bands were observed at 3570 and 622 cm^{-1} as a result of the stretching of hydrogen bond in OH ions present in HA and liberation mode of hydrogen-bond OH ions respectively. The band which occurs at 1027 cm^{-1} result from v3 PO_4 but the band at 532 cm^{-1} indicates the presence of v4 PO_4. The absorptions bands observed at 2878, 1662, 1582 and 1533 cm^{-1} are respectively attributed to methylene (-CH_2), amide I(C=O), amino (-NH_2), and amide II (-NH) groups in chitosan. Carbonate bands at 1375, and 848 cm^{-1} occurred due to the incorporation of carbonate in HA from CO_2 in air. Also, the FTIR spectra revealed the shifting of characteristic bands of amine (1660 cm^{-1}) and C-O (1592 cm^{-1}) in chitosan to lower wave number in the HA-chitosan nanofibre composite as a result of the interaction of bond between chitosan and HA due to the electrostatic interaction between $–NH^{3+}$ and PO_4^{-3} and likewise between C-O and Ca^{+2} which bind the nano composite together [17]. This interaction might be responsible for the improved mechanical strength of the composite scaffolds as compared to CH or HA alone. The interfacial bonding between the HA matrix and CH fibre plays a prominent role in determining the ultimate mechanical property of the composite. A strong interfacial bonding between the two phases enables the composite to attain better mechanical properties. With the exception of metallic materials, most materials derive their strength from their primary and secondary bond strengths. The bond between the CH reinforcement fibre and HA matrix enable stresses to be transmitted from the matrix to the fibre to enhance better mechanical property [57].

5. Conclusion

Chitosan (CH) nanofibre from crab shell and hydroxyapatite, HA, from limestone were successfully synthesized with the 30/70 composite being the optimum volume fraction. Mechanical characterization of different volume fractions of the composite was carried out to study the effects of increasing volume fraction of chitosan nano fibre on the mechanical properties of HA. In addition, surface characterization of the composite was carried out using Fourier Transform Infrared Spectrometry (FT-IR). Results indicated that Increasing volume fractions of chitosan nanofibre in the matrix of HA decreased the compressive strength (10.12 MPa), hardness (420.80 HV) and elastic modulus (0.1583 GPa) of HA while its tensile strength (173.9 MPa), bending strength (157.96 MPa) and fracture toughness (14.72 $MPa.m^{1/2}$) increased. The FTIR revealed that strong adsorption interaction between the NH_2 group and the primary and secondary –OH group of CH with Ca^{2+} (metal coordination interaction) of HA might be responsible for the improved mechanical property of the composite scaffolds. It was found that increasing chitosan volume fraction in chitosan nanofibre/HA composite results in increasing mechanical strength of hydroxyapatite; and hence, this composite will be a suitable material for load bearing application.

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