

PREPARATION AND CHARACTERISATION OF CHITOSAN/NANO HYDROXYAPATITE COMPOSITES

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ABSTRACT

Nano hydroxyapatite (Nano-HA) was synthesised and incorporated into water soluble chitosan (WSCH) to form WSCH/nano-HA composite using mixing technique. 30% glycerol was added to produce flexible composite. Film of WSCH/nano-HA composites was prepared using casting and drying method. The WSCH/nano-HA composite was characterized using scanning electron microscope (SEM), X-ray diffraction (XRD) and Fourier-Transformed Infrared spectroscopy (FTIR) to determine the morphology of the composites and to confirm the presence of hydroxyapatite in the composites. Results from XRD and FTIR confirmed the existence of HA in the composites while the SEM analysis indicated that hydroxyapatite was dispersed homogeneously in water soluble chitosan matrix. The mechanical properties of the composite were tested using Universal Testing Machine (UTM). It was found that increasing nano-HA content in the composite will decrease the tensile strength.

INTRODUCTION

Bone repair or regeneration is a common and complicated clinical problem in orthopedic surgery. Hence, ideal bone-substitute materials have been widely studied. Natural bone is actually an inorganic/organic composite mainly consisted of nano-structure hydroxyapatite (HA), $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$ and collagen fibers. HA is a major inorganic component of bone and one of the excellent candidates for bone repair and regeneration. This is owed to its similar chemical composition to that of the mineral phase of bone and hard tissues in human. Synthetic HA has been used extensively for biomedical implant applications and bone regeneration due to its bioactive and osteoconductive properties [1]. In addition, HA can accelerate the formation of bone-like apatite on the surface of the implant [2]. Owing to its brittleness, the instability of the HA particulate is often encountered when the particles are mixed with saline or patient's blood, making it unable to be used as bone regenerating template. To solve the problem, incorporation of HA into polymer matrix has been carried out to increase osteoconductivity and biodegradability with significant enhancement of mechanical strength. Chitosan (CS) can be utilized in combination with HA to meet these requirements. Chitosan is one of the most widely-used natural polymers in tissue engineering research that can be obtained by partially deacetylation of chitin which can be extracted from shells of crustacean (prawns, crab, squids, etc).

Many form of CS/HA composites have been synthesized such as powder form,

membrane, pastes, cements, micro sphere and scaffolds. The preparation of CS/HA composite has been reported elsewhere whether via mechanical mixing [3] or in situ precipitation [4] to produce chitosan/nano-HA composite. Nano size HA was found to enhance the growth of apatite on the composite scaffolds. In this study the nano-HA was synthesized and mixed with water soluble chitosan (WSCH) to form a WSCH/nano-HA composites.

EXPERIMENTAL DETAILS

Materials and Nano-HA preparation

Chitosan used was water soluble chitosan (WSCH) which is carboxylated chitosan (Shanghai Nicechem Co. Ltd). HA nanoparticles were synthesized in the laboratory using simplified technique [5]. The synthesis of HA was performed using $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ (System) and H_3PO_4 (Bendosen) with the Ca/P molar ratio maintained at 1.67. The synthesis was carried out by introducing H_3PO_4 into $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ solution in a drop-wise manner, which was kept stirred at slightly above room temperature and maintained at neutral pH using NH_4OH . After the synthesis completed, nano-HA suspension was aged for 48 hours. The precipitate was washed using deionised water, filtered, oven dried at 50°C and finally ground and sieved.

Preparation of WSCH/Nano-HA composites film

Nano-HA was added into 500 ml distilled water and ultrasonic for 30 min to disperse the nano particles. 10 g of WSCH was added into nano-HA suspension solution and stirred for overnight. About 30 % (w/w) glycerol was added to the prepared solution to act as plasticizer. The mixed solution was then cast into a PTFE mould and dry at room temperature to produce WSCH/nano-HA composites film. The percentage of nano-HA used in this study was 0 % (w/w), 10 % (w/w), 20 % (w/w) and 30 % (w/w).

Characterisation of Nano-HA and WSCH/nano-HA composites

Scanning electron microscope, (FESEM, LEO 1525) equipped with electron dispersive x-ray (EDX; Oxford) and Transmission Electron Microscope (TEM, Philip Tecnai 20, 200 kV) were used to evaluate the nano particles of HA. The chitosan film and porous scaffold was gold sputtered for 300 second and examined with SEM at accelerating voltage of 10 kV. To investigate the components of HA in WSCH matrix, the samples were analyzed using an X-ray diffractometer (Bruker). A Fourier Transform infrared (FTIR) spectroscopy, Perkin Elmer Spectrum 2000 FTIR spectrometer equipped with diamond tip HATR (Horizontal Attenuated Total Reflectance) accessories was used to study the structure of WSCH film and WSCH/HA composite film. For pure nano-HA powder, KBr pellet technique was used. HA powder was grinded together with KBr powder with mass ratio of 1mg HA in 300 mg KBr. The spectra were collected over the range of 4000 cm^{-1} - 550 cm^{-1} . The tensile properties of WSCH and WSCH/nano-HA composites were measured using a UTM (Tinius Olsen H5KS) at a cross-head speed of 10 mm/min. The sample films were cut into 1 cm x 9 cm strip and seven specimens were tested for each sample.

RESULTS AND DISCUSSION

Figure 1 shows the agglomeration of HA powders as observed using SEM. TEM micrograph of HA powder was shown in Figure 2 and the crystal structure of pure HA vision as rod-like in shape with a typical size of 70 - 100 nm in length and about 40 nm in width.

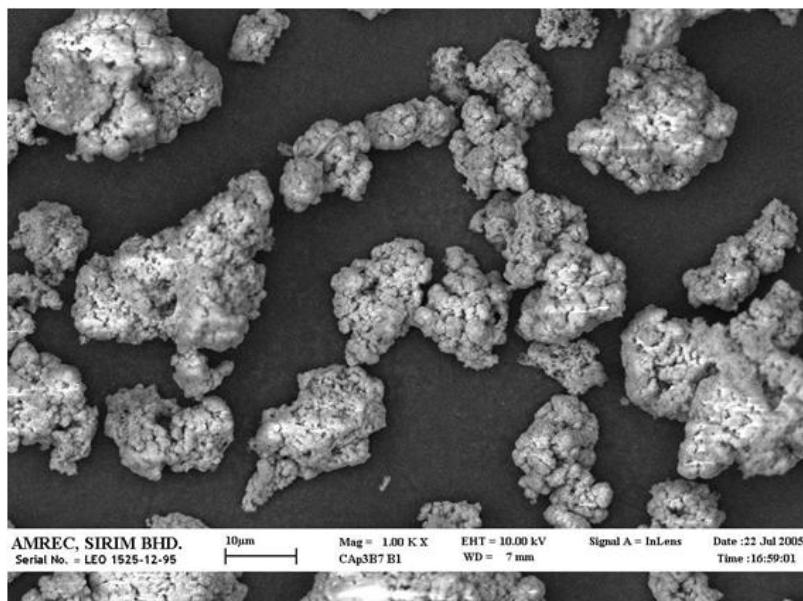


Figure 1: SEM micrograph of nano-HA powders

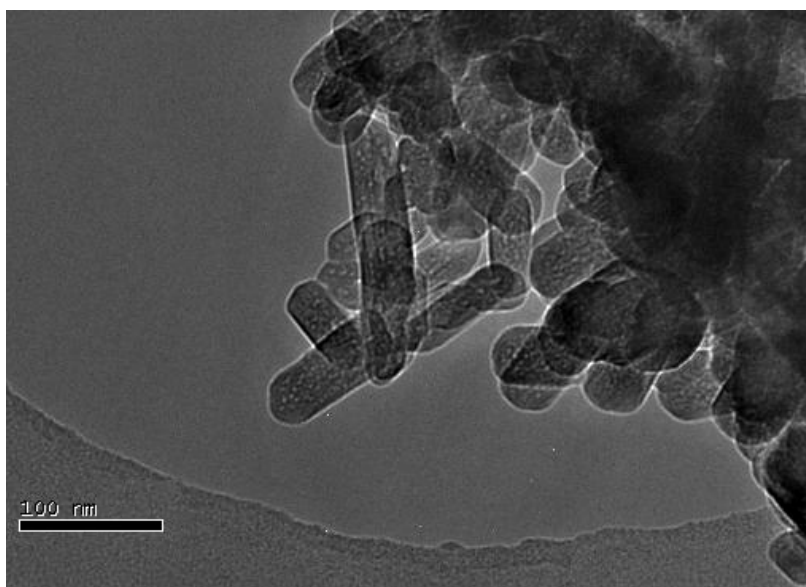


Figure 2: TEM micrograph of nano-HA powders

The surface of WSCH/nano-HA composites was analysed using SEM as shown in Figure 3. The HA powder was shown to disperse homogeneously in chitosan matrix and slightly bigger from original size (around 100 to 300 nm). This probably due to apatite mineralisation occurrence on the HA surface during mixing of the composites. HA nanoparticles resorb in chitosan solution and acted as nucleation site to enhance the deposition of apatite [6]. Electron dispersive x-ray (EDX) (Figure 4) confirmed that the mineral deposited on WSCH/nano-HA composites surface to be calcium phosphate. Calcium and phosphorus peaks are the elements that are present in HA while C is from WSCH.

FTIR analysis of the WSCH/nano-HA composites was complicated as the carboxymethylated chitosan and apatite band was overlapping. The FTIR spectrum of WSCH (Figure 5) shows bands at 3289 cm^{-1} belongs to vibration of hydroxyl groups while band at 1034 cm^{-1} , 998 cm^{-1} and 962 cm^{-1} are the characteristic bands of phosphate stretching vibration of hydroxyl apatite.

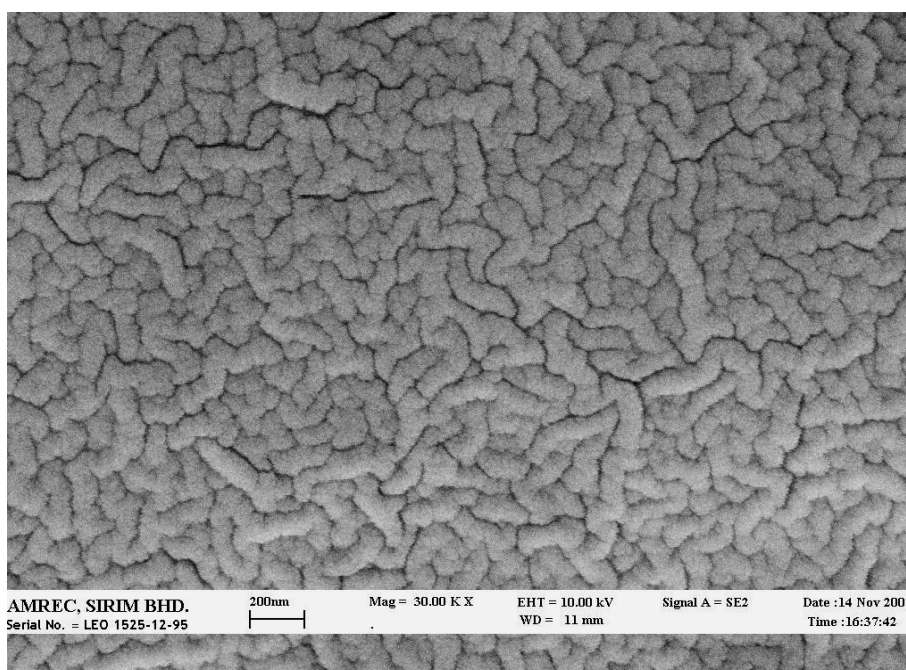


Figure 3. SEM micrograph of WSCH/nano-HA composites (10 % (w/w) HA)

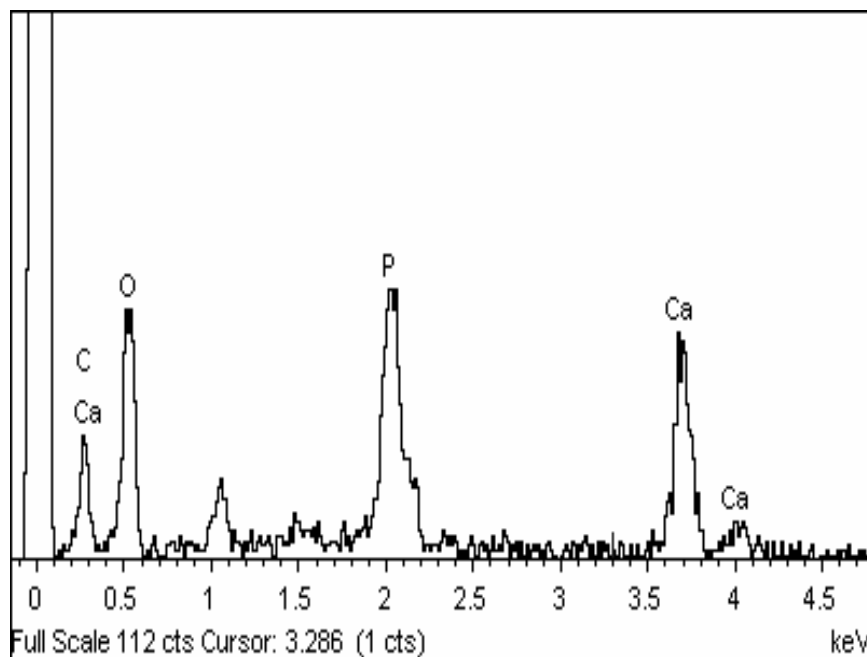


Figure 4. EDX spectra of WSCH/nano-HA composites 10 % (w/w) surface.

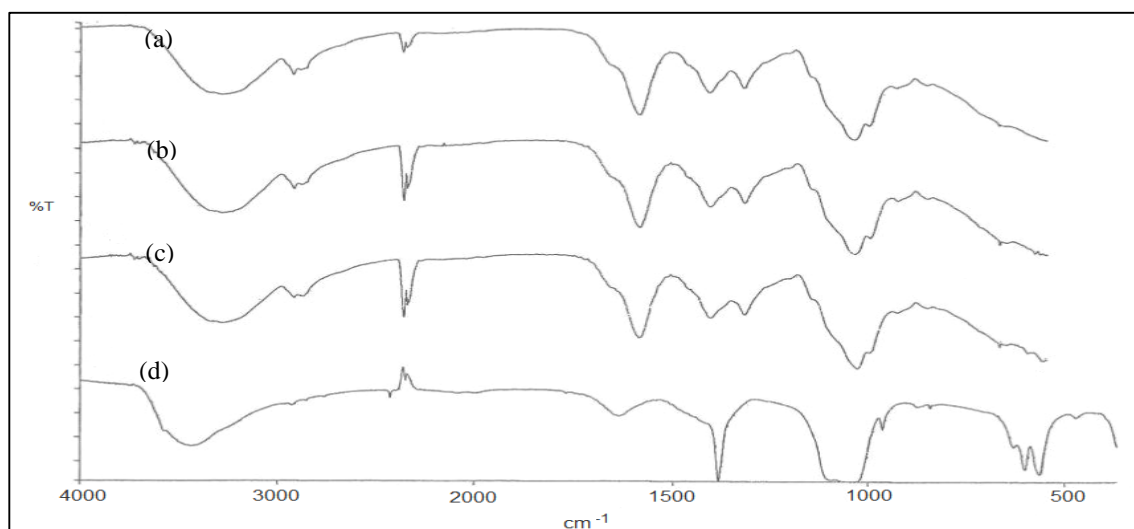


Figure 5: FTIR spectra of (a) WSCH film without nano-HA powder, (b) WSCH/nano-HA 15 % (w/w) film (c) WSCH/nano-HA 20 % (w/w) film (d) nano-HA powder

The XRD patterns of the chitosan and WSCH/nano-HA composites are shown in Figure 6. The peak appeared approximately at 21° was assigned to chitosan. The sharp diffraction characteristic peaks appeared at around 32° is the WSCH/nano-HA composites correspond to the peaks of HA powder. This results confirm the existence of HA compound in the chitosan matrix.

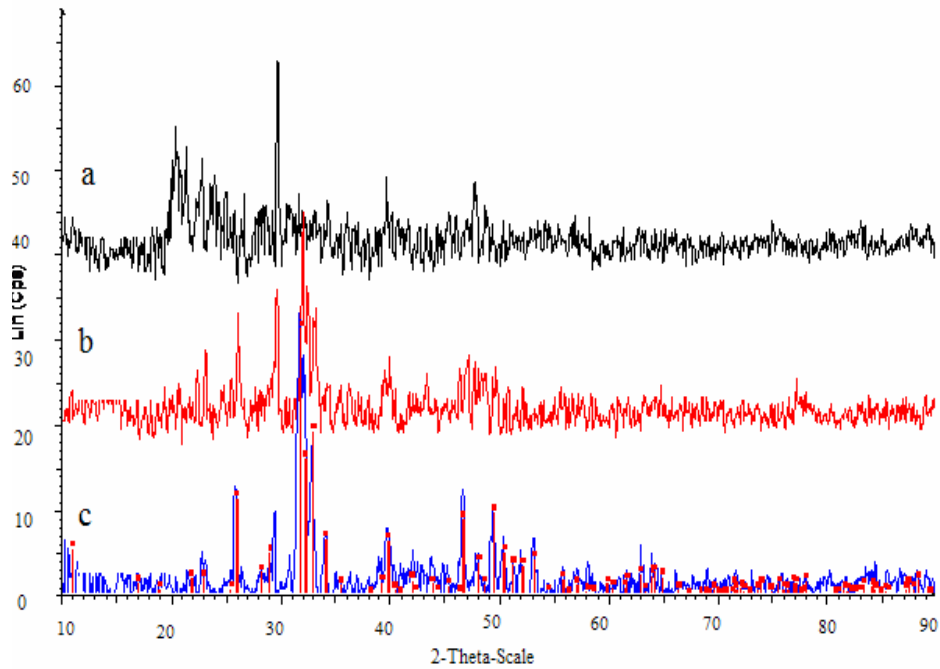


Figure 6: XRD patterns of (a) WSC film, (b) WSC/nano-HA 10 % (w/w) film and (c) nano-HA powder.

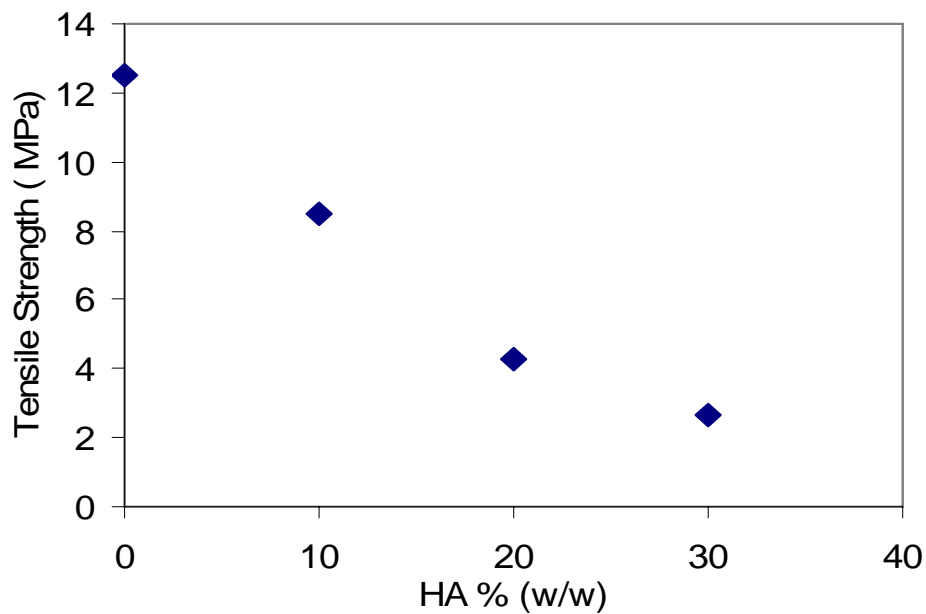


Figure 7: Tensile strength of WSCH/nano-HA composites as a function of HA content.

Figure 7 shows the effect of HA content on the tensile strength of WSCH/nano-HA composite. Generally the tensile strength was decrease as the HA content increased. Trend was observed where increasing the HA content decrease the tensile strength. The decrease of tensile strength is most probably due to weak interaction between HA particles and chitosan matrix. The WSCH/nano-HA with 10 % (w/w) was chosen as the optimum tensile properties of the composites. The strength of the WSCH/nano-HA composite is probably not suitable for load bearing applications but it is suitable for bone regenerating scaffold applications.

CONCLUSION

The HA powder synthesised in the laboratory was in nano size between 70 and 100 nm. The water soluble chitosan/nano hydroxyapatite composites were successfully prepared and the nano HA was dispersed homogeneously in water soluble chitosan matrix. It is found that the apatite mineralisation occurred during sample preparation. The WSCH/nano-HA composites have potential applications in medical such as bone regenerating scaffold.

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