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Research Article

Fabrication and mechanical properties of chitosan composite membrane containing hydroxyapatite particles

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Abstract: This paper described the development of chitosan composites containing precipitated hydroxyapatite particles for potential applications in orthopaedic surgery or waste water treatment. The synthetic process and morphology of hydroxyapatite were reported. The effects of hydroxyapatite content on the microstructure and mechanical properties of composites were investigated. It was found that the Young's Modulus of the composites decreases with hydroxyapatite content while the failure strength and strain increase with the hydroxyapatite content.

Key words: membrane; chitosan; hydroxyapatite; composites

1 Introduction

Chitosan is a natural fibre found in the crustacean's shells, insect's cuticle and cell wall of fungi [1-3]. Chitosan exhibits a unique set of physicochemical and biological characteristics, such as biocompatibility, biodegradability, antimicrobial activity, nontoxicity, physiological inertness, hydrophilicity, remarkable affinity to proteins, and high mechanical strength [4,5]. Hence chitosan has found numerous applications in various fields such as medical and pharmaceutical industries, tissue engineering, agriculture, nutritional enhancement and waste water treatment [3-8]. Chitosan is insoluble in neutral and alkaline condition. It is an excellent viscosity-enhancing agent in acidic environments due to its high molecular weight and a linear unbranched structure [5]. Viscosity also has an impact on biological properties such as wound-healing properties and osteogenesis enhancement as well as biodegradation [6].

Hydroxyapatite (HA, $Ca_{10}(PO_4)_6(OH)_2$) has been extensively used in medicine for implant fabrication owing to its similarity with mineral constituents found in hard tissue (i.e. teeth and bones) [9, 10]. Because of its high level of biocompatibility, it is commonly the material of choice for fabrication of dense and porous bioceramics [11]. Unfortunately, due to low mechanical property [12], sintered HA bioceramics

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cannot be used for heavy load-bearing bones. Thus, general usages include bioactive constituency in composites, coatings on metal implants and granular fillers for direct incorporation into human tissues [9-11]. Non-medical applications of HA include packing media for column chromatography, gas sensors, catalysts and host material for lasers [13-15]. Properties of HA, such as biocompatibility, solubility, castability, bioactivity, sinterability, fracture toughness and adsorption can be tailored over a wide range by varying particle composition (e.g. lattice substitution), particle size and morphology.

The current project was aimed to describe an inexpensive HA synthesis method focused on the precise control of particle size, morphology and chemical composition. Chitosan composites containing 20%, 30%, and 50% of HA were prepared at the different temperatures and pH to find out the optimum proportion of the hydroxyapatite/chitosan composite. The effect of the pH on the microstructure and mechanical properties was examined.

2 Experimental procedure

2.1 Precipitation of HA

The preparation of hydroxyapatite was by mixing of calcium chloride and ammonium dibasic phosphate at the desired ratio as described previously [16]. The pure hydoxyapatite (with formula $Ca_{10}(PO_4)_6(OH)_2$) has Ca/P=10/6=1.67. For this reason, in order to keep the right ratio between calcium (Ca) and phosphorous (P), and the calcium chloride was diluted to 0.2 mol/L and the ammonium dibasic phosphate was diluted to 0.12 mol/L. The dropping speed of the two solutions was maintained at the same value so that Ca/P= 0.2/0.12=1.67.

Each of the solutions was dropped into the reaction vessel, which was shown in the Fig. 1. Two different pH levels (pH7 and pH9) and two different temperatures, one was at room temperature of 20 ℃ and the other one was at around human body temperature of 40 ℃, controlled using a hot plate, and the magnetic stirrer was used to make the two solutions react uniformly and completely. The long burettes were used to make the reagents drop slowly, simultaneously in a controlled manner. The whole process of hydroxyapatite fabrication took about 60 min to ensure the reaction had taken place slowly.

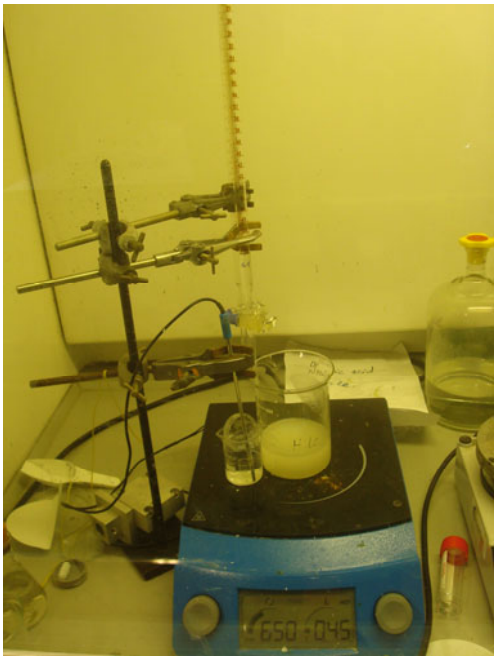

Fig. 1 Experimental setup for the precipitation of hydroxyapatite

Each of the beakers with formed precipitate was removed from the magnetic stirrer and placed in the water bath for aging at the same temperature (20 ℃ or 40 ℃) at which they reacted to form the precipitates. This is not only a way to make the calcium chloride and ammonium dibasic phosphate react completely, but also to make the hydroxyapatite deposit in the bottom of the beaker for further use.

According to the reaction equation, there were some chlorides in the beaker, which might affect the result of the test. It was removed by washing the precipitates by pouring the upper layer water out and then replacing with deionized water, this step was repeated three or four times to complete washing.

2.2 Preparation of chitosan

In order to prepare the chitosan gel, 2% chitosan (w/v) was placed into a beaker of deionised water. The beaker was on a magnetic stirrer, the speed of the magnetic stirrer was set at 600 rev/min and the temperature of ±1 ℃ can be achieved. In order to break down the chitosan and to make the chitosan dissolve into the deionised water uniformly, 2% acetic acid (v/v) was added to the beaker, and the solution was mixed for an hour and a half as described previously [17].

Table 1 Amount of HA added to chitosan

pH	Temp (℃)	Samples	Percentage of HA (%)	Weight of Pure Chitosan (g)	Weight of Pure Hydroxyapatite (g)
7	20	Pure CTS	0	2.0	0
7	20	CTS/HA	10	4.5	0.5
7	20	CTS/HA	20	4.0	1.0
7	20	CTS/HA	30	3.5	1.5
7	20	CTS/HA	50	2.0	2.0
9	40	Pure CTS	0	2.0	0
9	40	CTS/HA	10	4.5	0.5
9	40	CTS/HA	20	4.0	1.0
9	40	CTS/HA	30	3.5	1.5
9	40	CTS/HA	50	2.0	2.0

2.3 Mixing HA and chitosan

The pure hydroxyapatite must be prepared for mixing with the newly prepared chitosan. Acetic acid was added to the chitosan mixture which breaks down the chitosan and forms a uniform chitosan gel; the hydroxyapatite was added slowly and carefully into the gel; the speed and the temperature were controlled at the same time. The mixture was stirred for an hour and a half as the pure chitosan preparation process.

The solution should look uniform but different from the pure chitosan in colour, when the reaction of chitosan and hydroxyapatite completely finished. The samples for further testing were created in open moulds. Before pouring the chitosan/hyroxyapatite liquid gel, some cooking oil was smeared on the surface or the glass which would make the surface of the glass hydrophobic to aid removal of samples from the glass once samples were completely dried. With different series of the samples, the hydroxyapatite content did not affect the thickness of the specimens. In order to control the thickness (0.2 mm), 12 mL of gel were poured into a mould to give the specified thickness.

To avoid air bubbles, samples were agitated using shaking machine for about 10 min, this also aided uniform mixing of chitosan with hydroxyapatite. All other steps remained constant to ensure the samples were comparable.

The mixture was left 48 h at the room temperature to dry completely; samples were cured in a bath of 5% (w/v) sodium hydroxide (NaOH) for 20 min to fully cross link and form a stable membrane. Dried samples were removed from the moulds carefully to avoid fracture.

2.4 Mechanical testing

The composite material removed from the moulds was similar in appearance to wet paper, these were stored in the DI-water. They were cut into dog-bone shapes to allow for easy gripping during mechanical testing and to ensure fracture occurs in the central span of the material. The sheets of the material were placed on a sheet of PVC to be stamped, which preserved the sharp edge of the cutting stamp. Samples were tested using a Tinius Olsen H5KS bench top tensile testing machine, the loading rate was 30 mm/min.

3 Results and discussion

3.1 Form of results

The samples of each material were tested over several cycles in order to assess the repeatability of the properties in the materials, all the data from the different test processes are analyzed, and the results and curves are in the following.

3.2 Precipitates prepared at 20 ℃/pH7

It can be seen from the Fig. 2 that, the average stress changes with HA content, it is shown that, the average failure stress increases with increasing HA content, and it reaches a maximum when there is 30% HA in the material. It is believed that the addition of HA which improved the mechanical properties of the composites, however the mechanism of action is not yet fully understood.

The graph shows clearly that, more hydoxyapatite can provide a longer extension, which means the material will not easily break because of the effect from hydroxyapatite. The average strain also shows an increasing trend (Fig. 3).

Except for the average stress and strain, the Young's Modulus is a very important parameter for a material, it allows the deformation of a specimen to be calculated under certain load. For instance, it will be used to predict the amount a wire will extend under tension. Fig. 4 shows an obvious relationship between Young's Modulus and HA content. Apart from the pure chitosan, 30% of HA is the best choice of all the materials manufactured, because it has highest Young's Modulus.

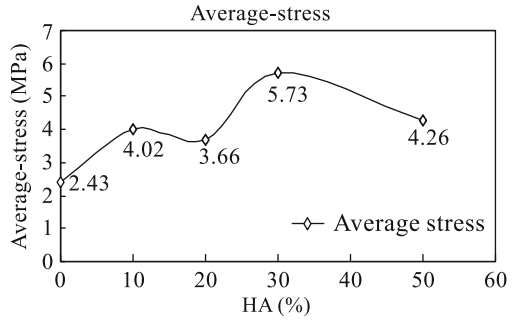

Fig. 2 Average failure stress of composites prepared at 20 ℃/pH7

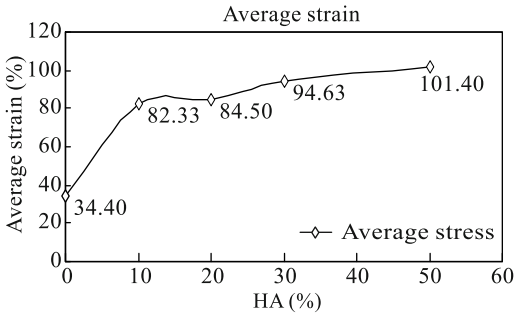

Fig. 3 Average failure strain of composites prepared at 20 ℃/pH7

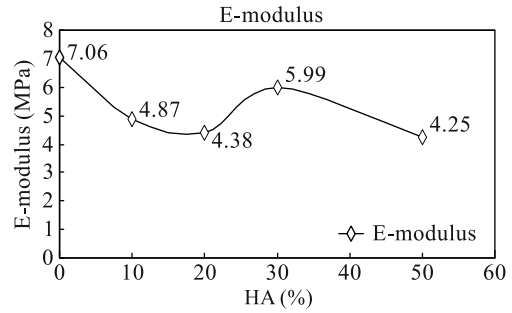

Fig. 4 Average Young's Modulus of composites prepared at 20 ℃/pH7

3.3 Precipitates prepared at 40 ℃/pH9

From Fig. 5 it could be seen clearly of the trends of average stress. It reaches the maximum point at 20% of HA instead of 30% under 20 ℃/pH7. The maximum average stress is 2.64 MPa. The difference between 20% of HA and 30% of HA is not that obvious, and the average stress of 30% of HA is around 2.52 MPa.

As shown in Fig. 6, the average strain is about the same at 30% and 50% HA, but they do not reach the maximum point. The maximum average strain occurs at the point of 20% HA, 71.77%. It is much higher than any others.

Similar to the samples of 20 ℃/pH7, the average Young's Modulus drops from the very beginning and

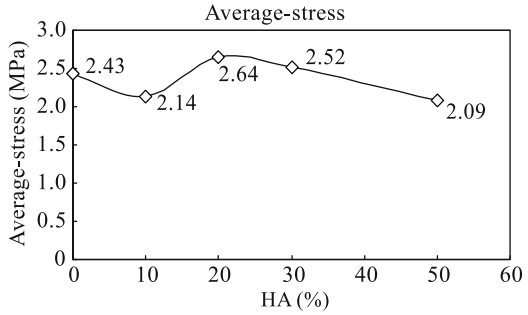

Fig. 5 Failure stress of composites prepared at 40 ℃/pH9

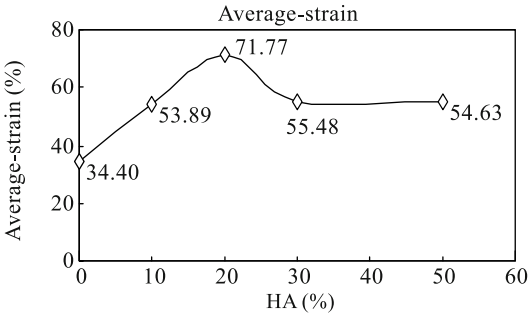

Fig. 6 Failure strain of composites prepared at 40 ℃/pH9

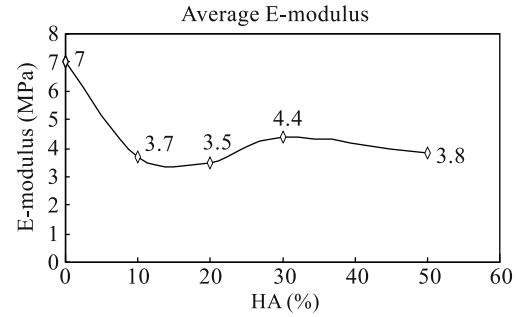

Fig. 7 Young's Modulus of composites prepared at 40 ℃/pH9

then it increases to the maximum point again when it is 30% of HA (Fig. 7).

3.4 Discussion about two different conditions of materials

The Young's Modulus comparison graph is shown in Fig. 8, each of the curves represents the average Young's Modulus of each of the precipitates that were prepared at two different pH levels and two different temperatures. The mean Young's Modulus of the pure chitosan was 7.06 MPa which is the highest of all the samples. Both curves exhibit the same trends with increasing amount of HA, it drops from the pure chitosan, and reaches a minimum value when there is 20% HA. Then the average Young's Modulus increases to a maximum at 30% HA. After this value it gradually decreases with increasing HA. The reduction in Young's Modulus with the addition of hydroxyapatite is inferring a poor interfacial bonding between chitosan molecules and hydroxyapatite particles.

Comparing results for pH9 with pH7, the average Young's Modulus of pH7 is higher than that of pH9, because the pH level is only controlled during the process of fabricating hydoxyapatite, therefore it is assumed that the morphology of the hydoxyapatite particles has an effect on the average Young's Modulus of the composite. The authors have reported that the crystals formed at neutral pH have plate-like shape which helps to reduce deflection so that the composite has higher Young's Modulus [16].

3.5 Microstructure study

Two images of 30% HA prepared at different pH levels were examined using SEM (Fig. 9). The microstructure varies considerably due to the different pH levels used in fabrication of the composite. From a closer view of the left image (fabricated at pH7), the plates are more scattered and the plates are typically interlocked, which can generate large inter particle forces and

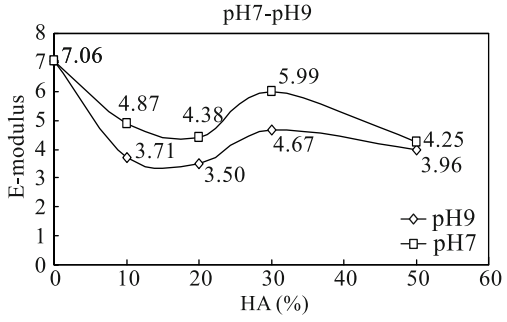

Fig. 8 Comparison of elastic moduli of composites prepared at pH7 and pH9

(a) pH7

(b) pH9

Fig. 9 Comparison of 30% at different pH levels, (a) pH7 and (b) pH9

friction when the specimen is undergoing tensile loading. Therefore the material shows a higher Young's Modulus than other composites.

Figure 9b indicates a regular plate like structure. The plates are smaller and have more cleavages than that in Fig. 9a. It is due to this microstructure, the Young's Modulus is not as high as that of pH7. The materials of pH9 may give a better dissolution into the real bone, its biological performance is better, but its mechanical properties are not as desirable.

From the above discussion and SEM images it can be concluded that the composite can be easily manufactured and some can match the mechanical properties of the natural bones. The composite can be used for soft bone scaffolds, and the mechanical properties have been improved compared to those of previous experiment.

4 Conclusions

The sol-gel process of a co-precipitation reaction was

adapted to fabricate hydroxyapatite. Two processing variables—the pH level and the temperature were investigated. From the research that was carried out, HA prepared at pH9 and 40 ℃ contains smaller plates and has more cleavages than that prepared at pH7 and 20 ℃. Composites containing HA prepared at pH7 and 20 ℃ exhibit higher Young's Modulus and failure stress than those containing HA prepared at pH9 and 40 ℃. This indicates the platelets precipitated under pH7 and 20 ℃ are stronger than those prepared at pH9 and 40 ℃.

No matter what the condition of HA fabrication is, the Young's Modulus of the chitosan composites is lower than pure chitosan. This infers that there is poor interface bonding between HA particles and chitosan. However, the composites containing 30% of HA particles exhibit an apex Young's Modulus. This is owing to the interlocking network of HA particles in the composites. The breaking strength and strain of the chitosan composites increase with the addition of HA particles. This indicates that the presence of mineral particles inhibit the relative sliding of the chitosan molecules so that the composite can sustain larger stress and strain. It is believed that this will improve the performance of the composite as bone tissue scaffolds or toxin absorbent in waste water treatment.

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