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Synthesis and characterization of carbonate hydroxyapatite

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Substituted apatite ceramics are of clinical interest as they offer the potential to improve the bioactive properties of implants. Carbonate hydroxyapatite (CHA) has been synthesized by an aqueous precipitation method and precipitates with two different levels of carbonate, processed as powders. Sintering experiments were performed to establish the influence of carbonate in significantly reducing the temperature required to prepare high-density ceramics when compared with stoichiometric hydroxyapatite (HA). High-temperature X-ray diffraction was used to characterize the phase stability of the apatites on sintering. Increasing carbonate content was shown to reduce the temperature at which decomposition occurred, to phases of CaO and β-TCP. Mechanical testing, performed using biaxial flexure, showed that the CHA specimens had strengths similar to stoichiometric HA.

1. Introduction

Hydroxyapatite (HA; $Ca_{10}PO_4)_6(OH)_2$) closely resembles the mineralized phase of bone and tooth, the structure of which corresponds principally to a crystallographic form of apatite [1]. HA is a bioactive material and when implanted *in vivo* it is able to bond with the host tissue by stimulating a specific biological response at the host/biomaterial interface.

However, biological apatites differ chemically from stoichiometric HA in that they contain a number of additional trace elements substituted into the HA lattice. One of the major substituents is carbonate (CO_3^{2-}) which in bone mineral occurs at levels, typically, of 5–8 wt % [2, 3].

Therefore, simulating the chemistry of mineralized tissue in bioceramic materials may be a means of improving the level of biological response of the implant to the host *in vivo* [4, 5]. In HA, this can be achieved by selectively substituting ions into the crystal structure during synthesis.

The wider aims of this study were to produce a range of carbonate-substituted apatites, that replicated the levels of carbonate found in biological apatites, and to assess their suitability as enhanced bioactive materials. The work presented in this paper seeks to characterize the effect of carbonate on the synthesis, processing and mechanical strength properties of this material.

2. Materials and methods

2.1. Synthesis and processing

The method of carbonate hydroxyapatite (CHA) precipitation used in this study was based on previously reported work involving the aqueous precipitation of both stoichiometric and carbonated HA [6, 7]. Analytical grade reagents (BDH AnalaR®, Merck Ltd, Lutterworth, UK) were used to prepare calcium nitrate 4-hydrate ($Ca(NO_3)_2 \cdot 4H_2O$) and di-ammonium hydrogen orthophosphate ($(NH_4)_2HPO_4$) solutions with concentrations in the ratio of 1 : 0.6 M. Two levels of carbonate substitution were achieved by adding 0.2 mol (CHA1) and 0.4 mol (CHA2) sodium hydrogen carbonate to the di-ammonium hydrogen orthophosphate solutions prior to reaction.

The precipitations of CHA took place over 2–3 h as the diammonium hydrogen orthophosphate solution was added dropwise to the calcium nitrate solution. Throughout the reaction, the pH was stabilized at $\geqslant 11$ using ammonium hydroxide solution and the temperature controlled at 20 °C. The resulting suspension was then aged for 24 h and washed thoroughly in deionized water.

To form powders, the precipitates were first filtered under vacuum. The dried filter cakes were then ground in a pestle and mortar before being ball milled for 90 min. The milled powder was then sieved for 60 min using BS410:86 sieves (Endecotts Ltd, UK), and only the powder which had passed through a 75 μm mesh was used for further experimental study.

2.2. Powder characterization

The particle-size distribution of the processed powders was assessed using laser diffraction with a Malvern Mastersizer X (Malvern Instruments, Malvern, UK). The specific surface area of the powders was measured by the Brunauer–Emmett–Teller (BET) gas

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adsorption method using nitrogen gas as the adsorbate (an analysis performed by Micromeritics Ltd, University of Surrey, UK).

The level of carbonate was determined as carbon using a CHN elemental analyzer (Medac Ltd, Brunel University, UK). X-ray fluorescence (XRF) (Ceram Research, Penkhull, UK) was used to determine the levels of calcium, phosphate, sodium and trace impurities in the unfired powders. The Ca/P molar ratio was calculated as a direct measurement from the XRF data while a Ca′/P′ ratio was calculated from the molar ratio of (Ca + Na/P + C), after Nelson and Featherstone [7].

To assess phase purity, X-ray diffraction (XRD) patterns were collected using a Siemens D5000 Diffractometer (Siemens, Munich, Germany) with CuK_α radiation ($\lambda = 0.154\,18$ nm) and phases present were compared with ICDD standards [8].

2.3. Sintering studies

To form green powder compacts, approximately 2.5 g powder was placed in a 30 mm diameter die and uniaxially pressed at ~200 MPa. Sintering was conducted between 800 and 1100 °C in a Carbolite STF 16/75 tube furnace (Carbolite, Sheffield, UK) under a moist carbon dioxide atmosphere This sintering atmosphere has been shown in previous studies to minimize carbonate loss from the CHA structure [9] and to promote densification [10]. The furnace was heated to the desired sintering temperature at 2.5 °C min^{-1} and, after a dwell period of 2 h, cooled at 10 °C min^{-1}. Carbon dioxide gas was bubbled through a flask containing approximately 200 ml distilled water before being introduced into the furnace. Sintered densities were measured by the Archimedes method and expressed as a percentage of the theoretical density of HA, assumed to be 3.156 g cm^{-3}.

2.4. Infrared spectroscopy

Fourier transform–infrared spectroscopy (FT–IR) was performed to determine the mechanism of carbonate substitution within powders heated under sintering conditions. A photo acoustic sampling technique was used to produce infrared spectra on a Nicolet 800 spectrophotometer between wave numbers 400 and 4000 cm^{-1}.

2.5. High-temperature XRD

The formation of decomposition phases at temperature was assessed using high-temperature X-ray diffraction. A coating of CHA was obtained on the platinum high-temperature stage (Edmund Bühler REP 1800, Bodelshaven, Germany) by placing a mixture of powder and ethanol and briefly heating to evaporate the solvent. The stage was then heated, at 5 °C min^{-1} under a CO_2 atmosphere to simulate sintering conditions and in air, and diffraction patterns were taken at 100 °C intervals between 500 and 1100 °C.

2.6. Mechanical characterization

Batches of HA and CHA discs were sintered at the optimum temperature for maximum density for mechanical testing in biaxial flexure. An Instron 4464 bench top test machine (Instron Ltd, High Wycombe, UK) fitted with a 2 kN load cell was used with a ring-on-ring flexure jig. The support ring of the jig was 17 mm diameter and the loading ring 4 mm diameter. The crosshead speed used was 0.5 mm min^{-1}. Maximum flexural strength was calculated from equations set out in ISO13356 [11]; Poisson's ratio for CHA was assumed to be 0.275, based on previous work on stoichiometric HA [12]; while Weibull moduli were determined using median rank values for survival probabilities.

3. Results

3.1. Powder characterization

The physical characteristics and chemical composition of CHA and HA powders are shown in Table I. The common processing route served to produce CHA powders with similar median particle size and surface area.

The molar Ca/P ratio in Table I is a direct measurement from the XRF data and reflects the replacement of phosphate ions in the crystal lattice by carbonate. The Ca′/P′ ratio is much lower than the ratio of 1.67 at stoichiometry. These results indicate that the mechanism of carbonate substitution was probably a combined sodium for calcium and CO_3 for PO_4. As the Ca′/P′ ratio assumes all the CO_3 substitutes for PO_4, it is possible that some of the CO_3 has substituted for OH groups, which would result in higher values of Ca′/P′.

X-ray diffraction traces showed powders of low crystallinity, represented by broad diffraction peaks, and free of secondary phases.

3.2. Infrared spectroscopy

The FT–IR spectra for CHA and HA are shown in Fig. 1. The effect of carbonate substitution can clearly be seen by peaks between 1410 and 1540 cm^{-1} and at approximately 880 cm^{-1} which are associated with the CO_3 ν_3 and ν_2 vibrational modes, respectively. The higher level of carbonate in CHA2 is reflected by an increase in the intensity of these peaks. An analysis of carbonate and phosphate peak assignments in the CHA spectra give evidence of a mainly CO_3 for PO_4 (B-type) substitution.

TABLE I Physical and chemical characteristics of CHA and HA powders

Sample	Surface area ($m^2\,g^{-1}$)	Median particle size (μm)	CO_3 (wt %)	Ca/P	Ca′/P′
CHA1	47.0	10.85	7.6	1.75	1.52
CHA2	52.9	10.31	10.2	1.91	1.56
HA	63.0	22.10	0.9	1.66	1.61

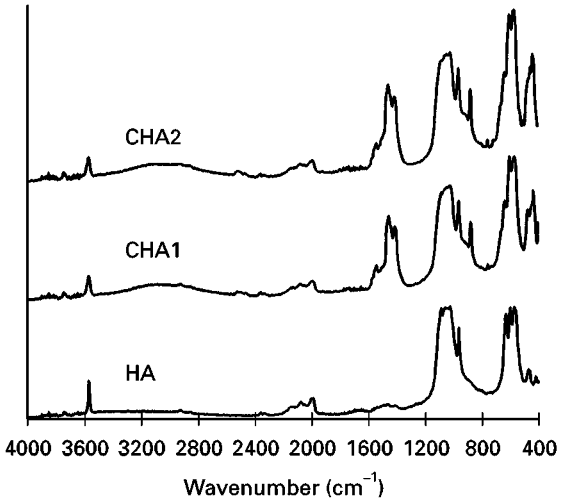

Figure 1 FT–IR spectra for CHA1 (CO_3 = 7.6 wt %), CHA2 (CO_3 = 10.2 wt %), and stoichiometric HA.

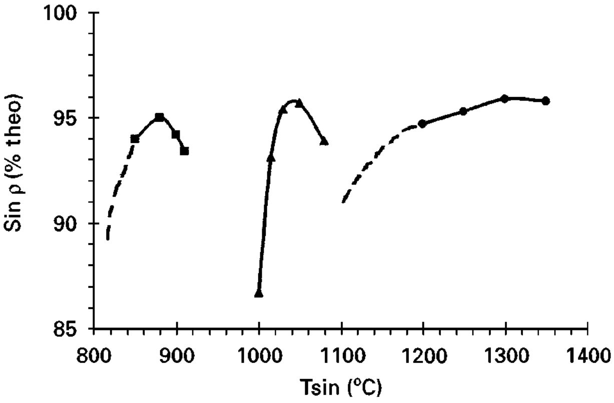

Figure 2 Sintering temperature versus density for (▲) CHA1 (CO_3 = 7.6 wt %), (■) CHA2 (CO_3 = 10.2 wt %), and (●) stoichiometric HA.

TABLE II Optimized sintering conditions for CHA and HA

Sample	Green ρ (%theo.)	Sintered *T* (°C)	Sintered ρ (%theo.)	Linear shrinkage (%)
CHA1	56.6	1030	95.4	21.1
CHA2	52.5	880	95.0	22.0
HA	55.0	1200	96.2	21.1

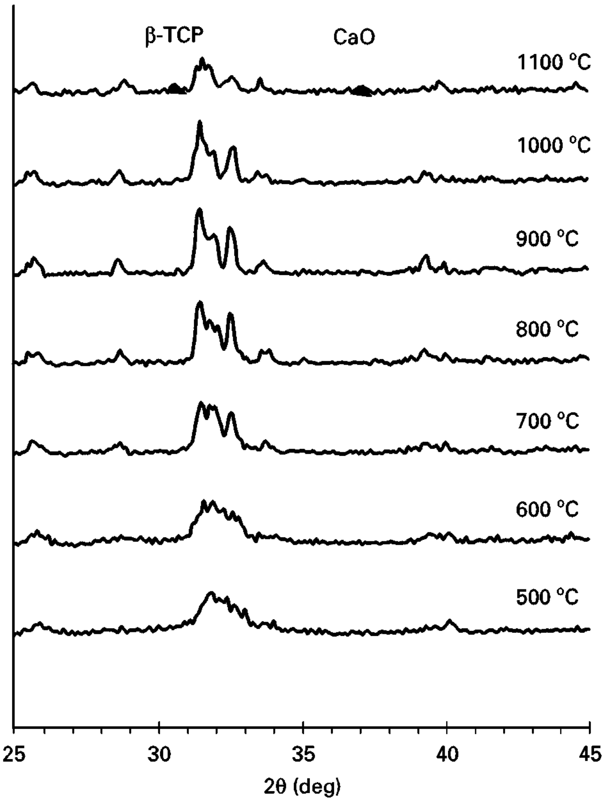

Figure 3 High-temperature XRD traces for CHA1 (CO_3 = 7.6 wt %).

3.3. Densification and decomposition

The results of the sintering studies are shown in Fig. 2. The level of carbonate incorporation in CHA1 and CHA2 significantly affected the temperature required to sinter to maximum density when compared to stoichiometric HA, prepared in a similar way. The highest level of CO_3 substitution, CHA2, required the lowest temperature to achieve a density of >95% theoretical. The optimum sintering conditions for both CHA compositions are shown in Table II.

Figs 3 and 4 show the results of the high-temperature XRD analyses for CHA1 and CHA2, respectively. The level of carbonate in CHA2 led to a marked increase in crystallinity at 700 °C when compared with CHA1. The influence of carbonate on phase stability is evident in the temperatures at which secondary phases are formed on decomposition. Peaks corresponding to phases of CaO and β-TCP were observed to form at approximately 900 °C in CHA2 but only began to appear at 1100 °C in CHA1. These temperatures are comparable to those encountered during sintering where decomposition was evinced by warping and bloating of the disc specimens. When the analysis was repeated in air, decomposition was observed to begin at significantly lower temperatures.

3.4. Mechanical characterization

The results of biaxial flexure testing are shown in Table III. The mean flexural strength of CHA2 at approximately 57 MPa (maximum strength ~74 MPa) was slightly lower than that of CHA1 (~64 MPa, maximum strength ~97 MPa). These values suggest a decrease in strength with increasing carbonate; however, CHA2 displayed a higher Weibull modulus. Both compositions showed flexural strengths comparable with stoichiometric HA prepared in a similar manner (mean ~66 MPa) with sintered density of 96% theoretical.

4. Discussion

The method of synthesis used in this study has produced CHA comparable, in terms of carbonate content and mode of substitution, with that of previous reports [7, 9, 13]. In producing ceramic CHA this report demonstrates the effect of carbonate on sintering conditions, phase stability at high temperature and the flexural strength of the material.

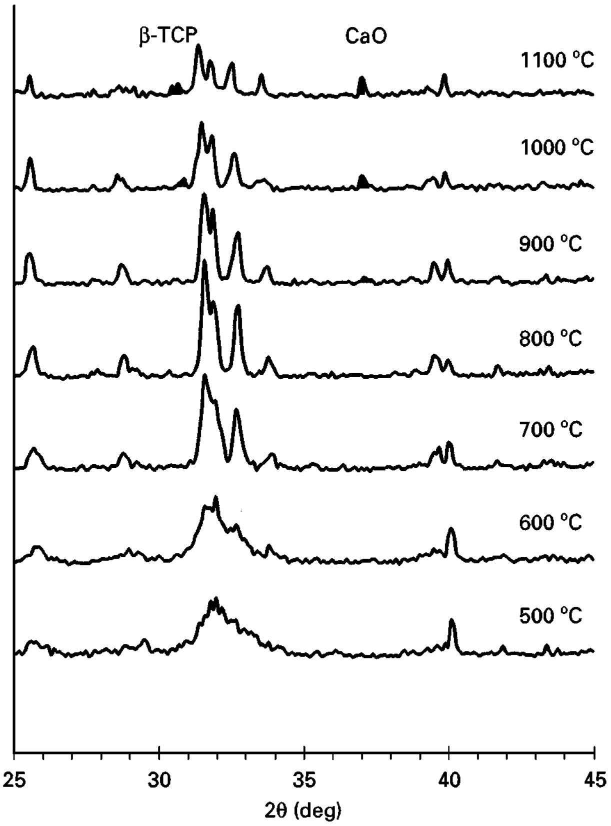

Figure 4 High-temperature XRD traces for CHA2 (CO_3 = 10.2 wt %).

The loss of carbonate from the CHA structure during sintering under a moist CO_2 atmosphere has been shown to range from 14–30% [9]. It can be assumed therefore, that the carbonate contents and mode of substitution, confirmed by infrared spectroscopy, of the sintered CHA ceramics produced in this study are of biological relevance.

Densification has been shown to commence at lower temperatures with increasing carbonte content in CHA and follows a similar trend to patterns observed in previous studies [9, 14]. A reduction in the required sintering temperature is clearly desirable in terms of ceramics manufacture; however, the formation of extraneous phases in a bioceramic can be detrimental in preparing materials of reproducible bioactivity. The exact nature of the decomposition mechanism in CHA is still unclear, but the high-temperature XRD analyses of this study have allowed the evolution of secondary phase to be observed *in situ* and enabled the sintering conditions required to produce single-phase CHA ceramics to be controlled.

This is the first report of the biaxial flexural strength of a sintered ceramic CHA and therefore there are no directly comparable data in the literature. The flexural strength of the CHA ceramics in this work were shown to be statistically similar to that for laboratory prepared stoichiometric HA. The number of test samples in each batch mean that the Weibull moduli quoted for each CHA composition have a sound statistical basis. The values reported here for strength and Weibull moduli lie within the range of previous studies, using similar test methods, for non-carbonate substituted HA [15, 16].

TABLE III Mechanical properties of CHA and HA

Sample	*n*	Flexural strength σ_F (MPa)	Weibull modulus, *m*
CHA1	28	63.53 ± 14.53	4.46
CHA2	31	57.04 ± 8.95	7.07
HA	10	65.9 ± 15	–

An analysis of the mechanical properties of CHA indicates a reduction in flexural strength with increased carbonate content. The potential benefits of enhanced bioactive response in a high carbonate content may be offset by decreased mechanical performance. Further work is needed to identify an optimal carbonate level that satisfies both mechanical and biological performance criteria.

5. Conclusion

This work has demonstrated that under a controlled atmosphere, single-phase CHA ceramics of physiologically similar carbonate contents with high densities can be produced at significantly lower sintering temperatures than those required for stoichiometric HA. The CHA ceramics tested were as strong in flexure and have the potential of enhanced bioactive response when compared to non-carbonated HA.

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