J Mater Sci: Mater Med (2008) 19:1187–1195
DOI 10.1007/s10856-007-3185-4

Effects of calcination temperature on the drug delivery behaviour of Ibuprofen from hydroxyapatite powders

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Received: 20 November 2006 / Accepted: 21 May 2007 / Published online: 15 August 2007

Abstract The effects of heat treatment time and temperature on the delivery behaviour of Ibuprofen from hydroxyapatite particles were investigated in this study. The drug release was seen to follow Fickian diffusion for the initial period of release for all heat treatment conditions. The gradient of Fickian release increased with (1) increasing crystallite size, attributed to the decreasing amount of boundary area, and (2) with decreasing surface area, due to the reduction in porosity and hence tortuosity within the apatite particles. This study has shown that altering the heat treatment conditions used to calcine hydroxyapatite may alter its drug delivery abilities, whereby calcination temperature was noted to influence the drug release behaviour to a greater extent than calcination time.

Introduction

The effectiveness of orthopaedic implants can be increased through designing substrates to allow for the slow release of therapeutic drugs, thereby minimising the induced inflammatory and possible infection response, and increasing the function of the implanted devices. It is desirable to limit this response by administering a drug at a controlled dosage level and rate at the site of infection or inflammation, thus reducing the required drug concentration and potentially minimising undesired secondary effects [1]. A method of improving treatment is the use of sustained released systems in natural and synthetic polymer and bioceramic implants, whereby the flux of a drug across a porous layer is dependant on two main parameters: the drug's solubility in the solution, and the possible physical or chemical interactions with the surface of the delivery device [2]. Traditional methods of providing drugs to the body are through oral or intravenous routes, which distribute the drug to all tissues, both healthy and ill, without discrimination [2]. Delivery systems that target the site where the drugs are required are therefore of great interest. Furthermore, site-specific drug delivery will help to avoid chronic bone infections such as osteomyelitis where limited blood circulation results in poor antibiotic distribution at the infection site making this condition difficult to treat [3].

Ibuprofen is a non-steroidal anti-inflammatory drug used extensively to treat bone diseases [1]. The drug is adsorbed onto the apatite surface, and its release dependant on its solubility in the medium used and the textural parameters from which it is released, whereby an increase in porosity, including the tortuosity of the pores, can alter the release behaviour. Ibuprofen release has been investigated from a number of substrates [1, 4, 5]. Studies by Ladrón de Guevara-Fernández et al. [1] into the release of Ibuprofen from composite samples comprised of SiO_2–CaO–P_2O_5 bioactive glass (to induce bone ingrowth), PLA (to facilitate drug release) and polymethylmethacrylate (PMMA) (to avoid instantaneous drug release) in simulated body fluid (SBF) showed that the rate of release was related to the growth kinetics of an apatite-like layer on the material surface, whereby a higher release of calcium into the SBF solution corresponded to higher release rate of Ibuprofen. Both the Ibuprofen release process and first stages of an

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apatite layer formation were recognised to be ruled by a diffusion process from the material into the SBF [1], however models explaining this process were not presented. Hydroxyapatite is a well-characterised material that is favoured for bone-related bio-applications due to its properties such as bioactivity, biodegradability and biocompatibility [6]. A wide variety of calcium phosphate materials are currently being investigated as potential drug delivery systems. Past attempts have used therapeutic agents including cephalexin and norfloxacin [7], aspirin [8], human growth hormone [9], anti-cancer drugs [10] antimicrobial silver [11], basic fibroblast growth factor [12], indomethacin [13–15], hydrocortisone acetate [2], and human serum albumin [16]. Hydroxyapatite has been investigated as a resorbable drug delivery device, whereby the mechanism of drug delivery could be fine tuned by changes in porosity. [2]. Investigations by Murugan and Ramakrishna [17] have shown that the release of neomycin from bovine hydroxyapatite was directly proportional to the porosity of the hydroxyapatite blocks, whereby the higher the porosity the greater amount of drug that was adsorbed and released. Despite the importance of such substrate characteristics, studies that determine the influence of other textural parameters such as the specific surface area or crystal size of hydroxyapatite powders on the release rate of active substances are lacking in the literature, and are of importance in the design of a drug delivery device.

In this study, the influence of calcination conditions on the textural parameters of hydroxyapatite powder and thus drug delivery behaviour were investigated. Specifically, hydroxyapatite powders were calcined at different temperatures and times, and the effects of altering the physical properties, such as crystallite size and surface area, on the drug delivery behaviour examined.

Materials and methods

Hydroxyapatite synthesis

Hydroxyapatite powder was synthesised by a precipitation method, whereby the addition of 1 L of a 0.6 M ammonium hydrogen orthophosphate (Riedel-de Haën, extra pure) solution to 1 L of a 1 M calcium nitrate 4-hydrate (Riedel-de Haën, analytical reagent) solution ensured sufficient reactants to produce stoichiometric hydroxyapatite according to the equation:

$$10Ca(NO_3)_2 \cdot 4H_2O_{(aq)} + 6(NH_4)_2HPO_{4(aq)} + 8NH_4OH \rightarrow Ca_{10}(PO_4)_6(OH)_{2(s)} + 20NH_4NO_{3(aq)} + 10H_2O_{(l)}$$

The reaction was controlled by a pH stat, specifically a 736 GP Titrino and 700 Dosino (Metrohm, Switzerland), using an ammonium hydroxide solution to maintain the pH at 9.4. The reaction was conducted in a polypropylene vessel supported in a water bath set at 37 °C, with a stirrer operating at 500 rpm and with (high purity) nitrogen gas purged through the solution to discourage the incorporation of carbonate from the atmosphere. After the reaction had reached completion (100 additions of 10 mL phosphate solution to the reaction vessel) the precipitate was left to age for 24 h to allow for the transformation from precursor phases such as amorphous calcium phosphate to the apatite phase. A filtration and rinsing process was then used to remove the waste solution. The suspension was initially filtered using a Buchner funnel, then rinsed twice with deionised water and re-filtered, rinsed in ethanol (to promote drying and produce a soft precipitate) and re-filtered, then dried in an oven at 60 °C for 24 h. Additional impurity ions such as ammonia and nitrates that remained after washing were removed by calcination.

Powder analysis

Thermal gravimetric analysis (TGA) was performed on a Setaram TG 92 with CS 92 controller, using a starting sample mass of $\sim$20 mg. The synthesised powder was heated to 950 °C in an air atmosphere at a rate of 10 °C/min to confirm suitable calcination temperatures.

Based on thermal gravimetric analysis results, 5 g batches of hydroxyapatite powder were calcined using a muffle furnace at 850 °C, 900 °C, and 950 °C for 15, 30, 60 and 120 min in a platinum crucible. Once the furnace had reached the desired temperature the sample was loaded into the furnace, which dropped a maximum of 20 °C from the target temperature for a maximum of 2 min. This was done to avoid the effects of temperature ramp-up on crystallite and particle growth to ensure that the results presented were specific to the time chosen and not the total time of the experiment. Once removed from the muffle furnace the powders were placed in a desiccator to cool down. After calcination the powders were fragile, and so were gently broken into particulate form. The powders were crushed with limited force to avoid inducing crystallinity which may potentially alter the drug delivery behaviour of the apatite.

Fourier Transform Infrared (FTIR) spectra were obtained using a Perkin Elmer Spectrum GX Infrared Spectrometer. 16 scans were collected for each heat treated powder between 4,000 cm^{-1} and 400 cm^{-1} with a resolution of 2 cm^{-1}. Discs were prepared by mixing approximately 1 mg of powder with 300 mg of KBr under vacuum at a pressure of 10 tons for 2 min.

Apatite powders were characterised by X-ray diffraction (XRD). Patterns were collected on a Geigerflex diffractometer (Rigaku, Tokyo, Japan) using Cu-Kα radiation

generated at a voltage of 40 kV and 22.5 mA using a scan speed of 1 s/0.05° between 2θ equal to 20° to 60°. Phase compositions analysis was performed using the Traces program Version 6.4.0 (Diffraction Technology Pty Ltd). Crystallite size was calculated for the (3 0 0) and (0 0 2) directions using the Scherrer equation.

An S-570 Hitachi scanning electron microscope (SEM) was used to examine the morphology of the apatite particles. The powders were adhered to aluminium stubs using carbon tape and then coated with a thin layer of gold using a sputter coater (Model SCD 005, Bal-Tech) at 25 mA for 180 s. SEM micrographs were recorded using an accelerating voltage of 10 kV at a working distance of 15 mm.

The specific surface area of each powder was measured using Micromeritics Gemini 2360 Brunauer-Emmett-Teller gas adsorption apparatus with nitrogen gas as the adsorbate. The saturation pressure of the gas was 760 mm/Hg, and the evacuation rate used was 200 mmHg/min. The samples were degassed overnight using a Micromeritics Flowprep 060 at 100 °C for dried samples and 350 °C for heat-treated samples. A silica-alumina standard (Micromeritics, GA, USA) was used before sample measurements to confirm the equipment calibration.

Drug delivery behaviour

To facilitate Ibuprofen loading onto apatite particles, a solution was prepared by dissolving Ibuprofen (Aldrich) in ethanol (BDH, analytical reagent) to form a concentration of 1 w/v%. 0.5 g of apatite powder was added to 25 mL of this solution and placed in an ES-20 Environmental Shaker PSU (Biosan, Riga, Latvia) where it was agitated at 150 rpm for 30 min at 37 °C. The solution was then poured over filter paper to collect the apatite particles, which were dried at 50 °C, well below the melting temperature of Ibuprofen (71 °C) [1].

The release of Ibuprofen was measured using a UV–Visible spectrophotometer (Varian). The spectrometer was calibrated using 5 standards at 37 °C, and the absorbance measured at λ = 264 nm. The samples were placed in 50 ml of pH 7.3 0.1 M tris buffer solution, and positioned in an ES-20 Environmental Shaker PSU (Biosan, Riga, Latvia) set at 100 rpm and 37 °C. The amount of drug released into the tris buffer was measured at 15 min after the initial immersion, then every 30 min for 4 h, and every hour for a further 3 h.

The release profile of Ibuprofen from the calcined powders was examined to determine whether it followed Fickian diffusion behaviour, whereby the release data was plotted according to [18]:

$$\frac{M_t}{M_\infty} = Kt^n \quad (1)$$

where M_t/M_∞ is the fractional solute release, K is a constant, t is the release time, and n is the diffusional exponent characteristic of the release mechanism, for which Fickian diffusion is defined by n equal to 0.5, and non-Fickian by n greater than 0.5 [18].

Results

Powder analysis

TGA was conducted to determine suitable calcination temperatures. The thermal gravimetric curve of hydroxyapatite powder is presented in Fig. 1, and shows three distinct gradients. The first is attributed to evaporation of adsorbed water, and the second to loss of nitrates, ammonia and other impurity ions remaining from synthesis. Weight loss then continues to decrease beyond 550 °C (third gradient), whereby relatively minimal mass loss is observed at temperatures in excess of 850 °C. This was therefore selected as the minimum calcination temperature, and increments of 50 °C were chosen to ensure large enough differences in results. A maximum of 950 °C was enforced due to restrictions of the furnace.

FTIR spectra of the powders calcined at 900 °C and an uncalcined reference sample are presented in Fig. 2 (similar trends were observed for all temperatures). The crystallinity of the samples increased following heat treatment, as evidenced by the distinction of the doublet phosphate peaks at 600 cm^{-1} and 560 cm^{-1} [19]. Water loss from within and on the structure of apatite crystals was represented by the decreased intensity of the peaks at 1,620 cm^{-1} and 3,420 cm^{-1}. Also, the loss of the sharp band at 1,384 cm^{-1} implied the removal of nitrate impurities remaining from synthesis.

Slight carbonation of the hydroxyapatite sample was shown by the presence of small carbonate v_2 vibrational modes at 866 cm^{-1}, due to labile carbonate groups [20], and v_3 at 1,450 cm^{-1}. These peaks were also removed with increasing calcination temperature.

XRD patterns shown in Fig. 3 indicate that each of the powders consist of a single apatite phase, matching to the 74–565 card for hydroxyapatite in the Traces Program Version 6.4.0 (Diffraction Technology, Pty, Ltd). Furthermore, additional phases such as β-tricalcium phosphate ($2\theta \sim 31°$) and calcium oxide ($2\theta \sim 37.5°$) were not observed. Crystallite sizes of the heat treated powders are reported in Table 1, where an increase with increasing calcination time and temperature can be observed.

SEM images of hydroxyapatite powders heat treated at 900 °C are presented in Fig. 4. Calcination time does not appear to have a significant influence on particle size, and furthermore each of the samples are observed to consist of powder agglomerates.

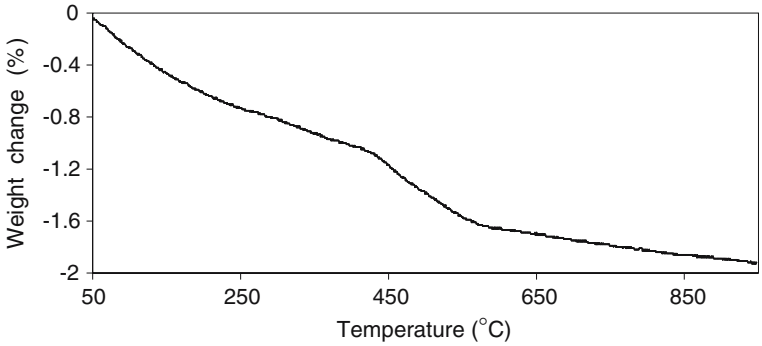

Fig. 1 Thermal gravimetric analysis curve of hydroxyapatite in air

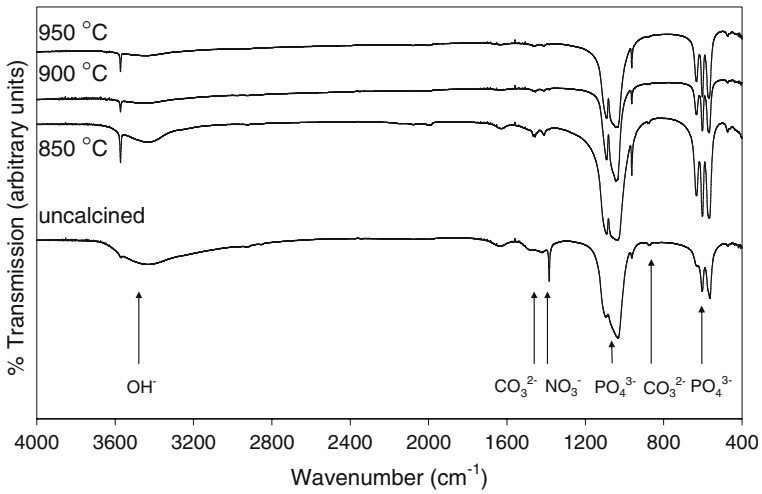

Fig. 2 Fourier Transform Infrared spectra of hydroxyapatite powders heat treated at 850 °C, 900 °C and 950 °C for 60 min

Surface area measurements, presented in Fig. 5, show a decrease in surface area with increasing calcination time and temperature. The largest decrease occurred between the uncalcined powders (60 m^2/g) (not represented on the graph for clarity at higher calcination times) and those calcined for 15 min; any additional decrease beyond this was small in comparison. The difference between 15 min and 30 min was most noticeable for 850 °C calcination, and less so at the higher temperatures. The gradient of each time increment then continued to follow this trend, decreasing with increasing temperature. For each heat treatment time, a difference of at least 2 m^2/g per 50 °C increase in temperature was noticeable.

Drug delivery behaviour

The average amount of Ibuprofen adsorbed by the calcined samples was 2.0×10^{-3} M with a standard deviation of 0.43×10^{-3} M.

Equation 1 was plotted in Fig. 6 to determine the time period over which a linear relationship, representing Fickian diffusion, exists. A comparison of the effects of calcination temperature showed that Fickian diffusion behaviour existed for the first 3.5, 2.0 and 1.3 h of release when calcined at 850, 900 and 950 °C for 60 min, respectively, and that the gradient of this release increased with increasing calcination temperature. Additionally, powders calcined at 950 °C for 15, 30, 60 and 120 min were all observed to follow Fickian diffusion behaviour for the first 1.33 h of release, and the gradient of release increased with increasing calcination time.

Discussion

Yu et al. [21] investigated the release of cephalexin and norfloxacin from self-setting hydroxyapatite cement and found that the initial rate of release could be described using the Higuchi equation [22]. Similar results were found by Otsuka et al. [14] for the release of indomethacin. Studies by Martins et al. [23] into the release of the antibiotic ciprofloxacin from anionic collagen/HAP composite paste have shown that the release was characterised by two steps, the first of which followed Fickian diffusion during the first 5 h and represented 15% of the drug release, the second corresponding to a further 16.8% release over the next 7 days. These studies confirm that the theoretical

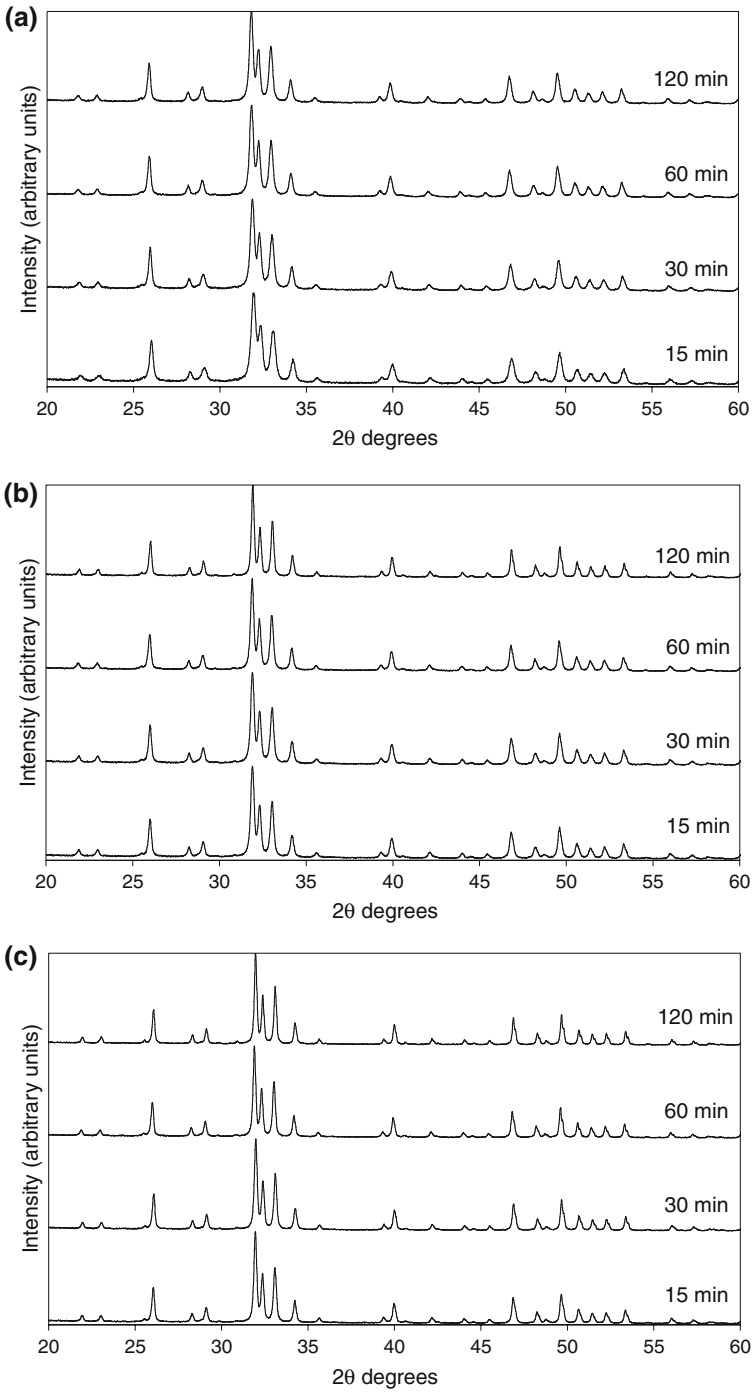

Fig. 3 X-ray diffraction patterns of hydroxyapatite powders heated treated at (**a**) 850 °C (**b**) 900 °C and (**c**) 950 °C for 15, 30, 60 and 120 min

analysis and models of drug release typically used to describe release from polymers can also be used for ceramic substrates.

Calcination is a form of heat-treatment that is used for apatite synthesised by precipitation methods to remove excess water and other impurities such as nitrates and ammonia that remain after washing. The resultant powder is not only of a purer form, but the applied heat causes crystallite and grain growth, thereby reducing the overall boundary area of the sample. Furthermore, heating to an elevated temperature for a significant period of time may also cause sintering, thus reducing the porosity of the material and significantly decreasing surface area as neighbouring particles agglomerate. The effects of altering heat treatment conditions on the drug delivery capabilities of hydroxyapatite granules were examined by Seshima et al. [24] using bisphosphonates. Changing the sintering temperature was observed to alter the surface morphology,

Table 1 Crystallite size of hydroxyapatite samples heat treated at different temperatures

Calcination		Crystallite size ($\times 10^{-10}$ m)	
Temperature (°C)	Time (min)	*a*-axis	*c*-axis
850	15	647	948
	30	854	1,315
	60	952	1,359
	120	1,062	1,482
900	15	1,033	1,509
	30	1,295	1,735
	60	1,454	1,631
	120	1,479	1,896
950	15	2,302	2,265
	30	2,511	2,548
	60	3,315	2,912
	120	4,875	4,077

specific surface area and crystallinity of the granules, thereby confirming that the sintering temperature used can control the physical properties of hydroxyapatite. Furthermore, it was shown that both crystallinity and surface area directly influence the total amount of bisphosphonate released over a 24 h and 72 h period, however models describing the release behaviour during this time were not presented.

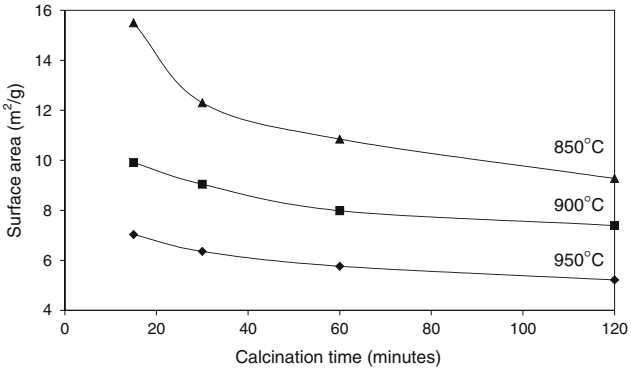

Fig. 5 Surface areas of hydroxyapatite powders heat-treated at different times and temperatures

Particulate forms of apatites have increased bioavailability, a predictable therapeutic response, a controlled and prolonged release time, and greater efficacy and safety than larger sized substrates [25]. The crystallite size of the hydroxyapatite particles was expected to play a role in determining drug delivery behaviour. The regions of

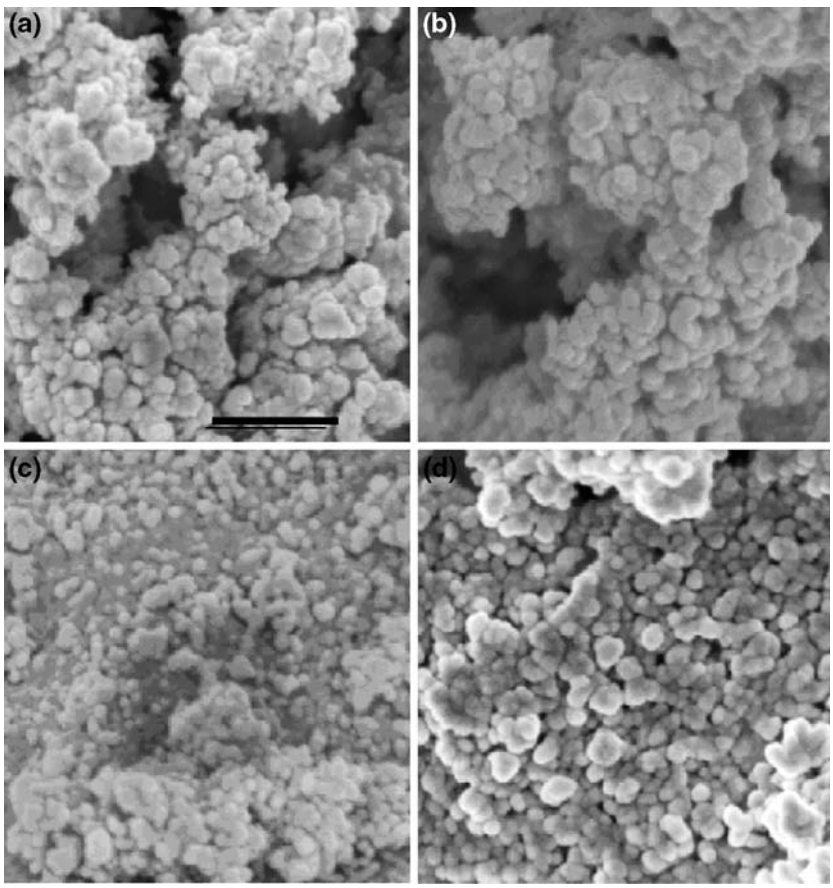

Fig. 4 Scanning Electron Microscopy images of hydroxyapatite heat treated at 900 °C for (**a**) 15, (**b**) 30, (**c**) 60 and (**d**) 120 min (scale bar 1 μm)

atomic disorder between neighbouring crystals of differing orientation represent regions of higher energy when compared to the highly ordered crystal, making these regions more chemically reactive [26]. This should allow a sample with smaller crystallites, and hence a larger area of disordered regions, to adsorb more drug than a sample with large crystallites. Additional effects of heat treatment will be the alteration of porosity, and hence permeability and/or tortuosity, of the particles, which may influence the period of drug release.

As the thermal gravimetric curve of hydroxyapatite powder shows a relatively minimal mass loss at temperatures in excess of 850 °C, it was therefore assumed that the majority of the impurities had been removed at the temperatures investigated to produce a pure powder for examination. Furthermore the hydroxyapatite phase was maintained for each sample, whereby thermal stability was implied by the relatively flat gradient of weight loss in this temperature region.

The peaks observed in FTIR spectra show that a relatively crystalline hydroxyapatite sample was produced, which increased crystallinity with increasing heat treatment temperature. Slight carbonation of the uncalcined sample was observed, indicating that this chemical substitution occurred during synthesis despite purging the reactant solution with nitrogen gas. The intensity of these peaks decreased with increasing heat treatment temperature, suggesting the decomposition of the carbonate present into CO_2 and OH^- according to:

$$CO_3^{2-} + H_2O \rightarrow CO_2 + 2OH^-$$

which may also contribute to the increase hydroxyl band intensity.

The maintenance of the hydroxyapatite phase with increasing heat treatment time and temperature was confirmed from XRD patterns, whereby additional components resulting from phase changes were not observed. The main change noted from examination of these peaks is an increase in sample crystallinity. The crystallite sizes of particles calcined at one temperature for 120 min are less than if calcined at a temperature 50 °C higher for only 15 min. The effects of an increased temperature of 50 °C therefore appears to be greater than the effect of increased time on atomic diffusion and hence crystallite growth within the apatite crystals studied. Therefore, it is expected that as calcination time and temperature are increased the increase in crystallite size and subsequent decrease in

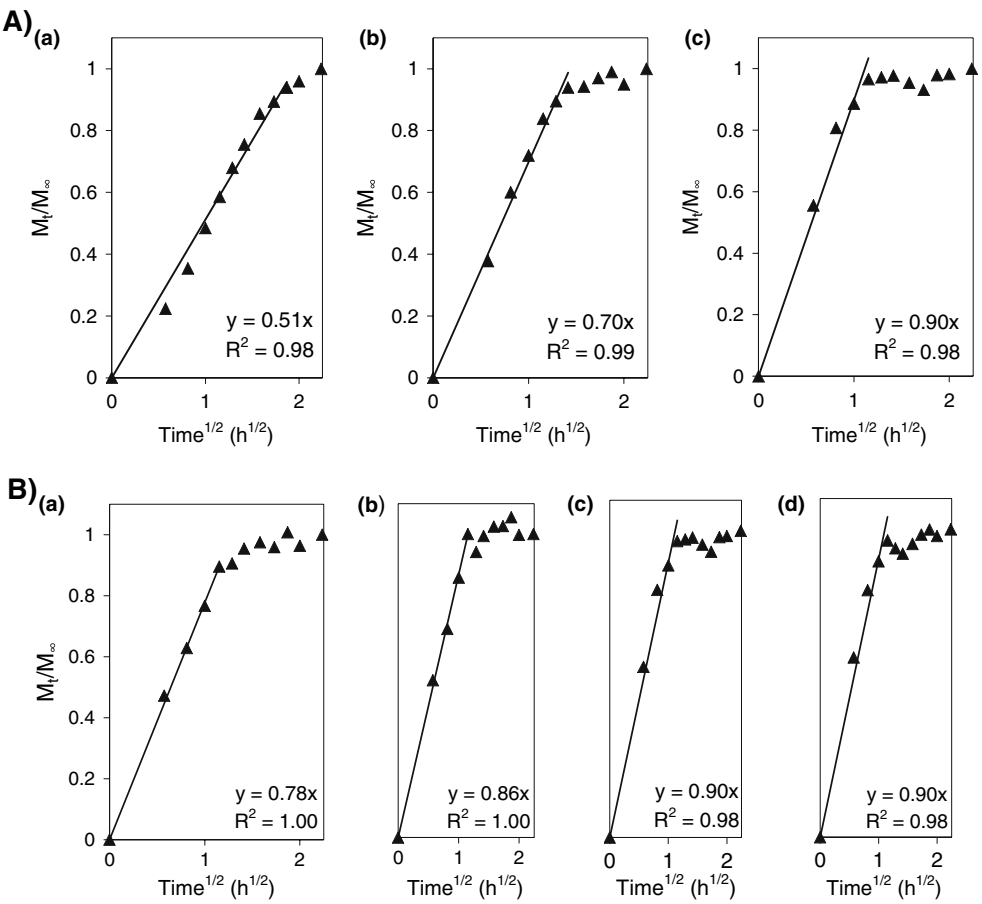

Fig. 6 (**A**) Fickian diffusion behaviour of Ibuprofen release (M) from hydroxyapatite powders heat treated for 60 min at (a) 850 °C (b) 900 °C and (c) 950 °C. (**A**). Fickian diffusion behaviour of Ibuprofen release (M) from hydroxyapatite powders heat treated at 950 °C for (a) 15 (b) 30 (c) 60 and (d) 120 min

boundary area would produce a sample with less chemically reactive surface sites to adsorb Ibuprofen.

The reduction in the measured surface area of the particles is attributed to both neighbouring particle agglomeration as well as a reduction in porosity, as observed in SEM images. Microporosity that exists within the particles could alter the drug delivery behaviour; however closed porosity within the particles due to particle sintering during calcination would not be a contributing factor as these pores do not come into contact with the drug during loading. Any open pores within the particles that are interconnected with the particle surface; or rather those that could potentially adsorb and later release the drug will be accounted for during surface area measurements.

Although acknowledged to affect the surface area and sintering of hydroxyapatite, the main effects of the particle size and distribution on drug delivery behaviour relates to the effects of the surface area available to adsorb the drug and the consequential release behaviour. Such changes due to heat treating the powders have been described through the reporting of changing surface area from BET measurements after each calcination temperature and time, and so this variable has been accounted for though an alternative, and more relevantly descriptive, measurement (BET).

The specific surface areas of the samples were noted to decrease dramatically following 15 min of heat treatment. Additionally, the surface area curves plateaued at different times for each temperature. The value of this plateau is not constant for HAP, suggesting that the minimum surface area achievable is dependant upon the calcination temperature used, and not the time. Fig. 5 shows that a similar surface area is achieved for a sample calcined at 900 °C for 15 min as a sample at 850 °C for 120 min, and a sample at 950 °C for 15 min as a sample at 900 °C for 120 min. However, as the crystallite sizes produced are smaller following calcination for 120 min at the lower temperature, the samples calcined at higher temperatures for the reduced time are likely to possess fewer sites suitable for drug adsorbance, despite having a similar surface area.

The release of Ibuprofen from the heat treated hydroxyapatite powders can be described by a diffusion process in the pores of the apatite particles, and therefore the release rate is dependant on the porosity and tortuosity of the pores in or between the sintered apatite particles over this initial linear release [21, 23], and hence will change correspondingly to pore size and shape.

It is important to note that any relationships between drug delivery behaviour and calcination temperature are not related to the calcination temperature itself, but the effects of this temperature on the sample's characteristics. During heat treatment the apatite sample will undergo structural refinements on three levels:

1. changes within the crystal structure, including a loss of water and impurities,
2. an increase in atomic diffusion representing an increase in crystallite size, and
3. agglomeration/sintering of particles and a loss of open porosity representing a decrease in surface area.

The first point is not expected to alter the drug delivery behaviour of the samples to a significant extent, as the temperatures used were greater than that required to cause water and nitrate removal for all samples as seen in Fig. 1.

In order to see any relationships clearly, the crystallite size (*a*-axis) and surface areas of the samples are listed in Table 2 with the value of the constant K describing the gradient of release. A clear correlation was seen when comparing the gradient of release during Fickian diffusion to both the crystallite size and surface areas of the samples. As the crystallite size increased, and hence the boundary region decreased, the gradient of release was seen to increase. This is believed to be due to Ibuprofen that is adsorbed onto the crystallite region of the particle not being as strongly attached compared to that adsorbed onto the boundary region, and hence as the proportion of the more weakly attached drug increases, the overall rate of release also increases.

As the surface area decreased with increasing heat treatment temperature, the initial rate of release increased. This decrease in surface area represents not only agglomeration or partial sintering of neighbouring particles, but a loss of porosity from inside the particles. The loss of porosity would reduce the tortuosity of the path the dissolved drug must take to move out of the particle into the surrounding buffer; hence as this porosity is decreased the gradient of drug released into the medium will be larger, and furthermore the period over which diffusion-controlled release exists will be reduced.

The trends noted between crystallite size and surface area and heat treatment conditions were observed for both changes in time and temperature. However, based upon the results presented here, heat treatment temperature had the

Table 2 Comparison of values of constant K to crystallite size and surface area for heat treated hydroxyapatite samples

Calcination conditions		K ($h^{-1/2}$)	Crystallite size (*a*-axis) ($\times 10^{-10}$ m) (±30)	Surface area (m^2/g) (±0.2)
Temp (°C)	Time (min)			
850	60	0.51	952	10.9
900	60	0.70	1,454	8.0
950	60	0.90	3,315	5.7
950	15	0.78	2,302	7.0
950	30	0.86	2,511	6.4
950	120	0.90	4,875	5.2

greatest influence on the change in the gradient of diffusion-controlled release, and correspondingly reduced the time period over which this process occurs. Both variables may therefore potentially be altered to produce a drug releasing substrate that has a very precise rate of diffusion-controlled drug release.

Conclusions

The surface area and crystallite size of hydroxyapatite powders can strongly influence the level of drug released, due to the differing drug adsorption-release characteristics of the crystalline versus boundary regions, as well as the effects of the tortuosity of the pore network that the drug must diffuse through.

In conclusion, this investigation has shown effective ways to alter the drug delivery behaviour of hydroxyapatite via changes in the physical characteristics induced through heat treatment, thus allowing the rate of delivery to be tailored to specific requirements.

Acknowledgements A. J. Melville would like to thank the Monash University Postgraduate Publication Award scheme for funding during the preparation of this manuscript. L. M. Rodríguez-Lorenzo is supported by the Ramon y Cajal program, Spain. This work was supported by a Monash Research Fund grant and Australian Research Council grant F10017027 and A10017174.

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