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A Method for Avoiding the Xanthoproteic-associated Discolouration in Reprecipitated (Nitric-acid-digested) Hydroxyapatite Prepared from Mammalian Bone Tissue

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A procedure for producing kilogram quantities of a biomedically suitable reprecipitated hydroxyapatite (HAp) powder, which is free of the xanthoproteic-associated discolouration caused by nitric acid interaction with the protein content in bones during digestion, has been developed. Essentially bones were defatted by boiling and then pyrolysed at 1000 °C to burn off the collagenous proteins and produce flakey bleached bones. This bone was then crushed and ground and digested in nitric acid solution to produce digest solutions free of the highly staining orange colouration normally associated with nitric acid digestions of bone material.. Finely divided, white solids could then be reprecipitated as usual from the digests by addition of NaOH solution under a nitrogen atmosphere with heating and stirring at 70 °C of the precipitate to enable its maturation into an HAp phase. The products derived from this procedure were characterised using spectroscopic, microscopic and particle sizing techniques. These analyses showed the resultant powders to be low crystallinity hydroxyapatite of consistent morphology and which possessed either acceptably low or below detection limit levels of heavy metals so rendering it as a potentially valuable source of powder for biomedical applications such as plasma spraying or for implant or prosthesis manufacture.

Key words
hydroxyapatite
mammalian bone
pyrolysis
reprecipitation
xanthoproteic discolouration
biomedical

INTRODUCTION

Meat by-products (described as the »remainder of the animal« once the edible meat is removed) account for 55 % of animal weight at slaughter, but represent only 24 % of the value of the carcass. Bone accounts for about 16–24 % of the carcass weight.[1] Given the current worldwide drive to be more environmentally accountable and economically efficient in the processing of raw products, achieving added value out of meat products by developing alternative high tech uses for the traditional by-products such as bone is an important priority. The potential »high tech« uses for bone outside its current applications as a bone meal for fertiliser would allow it to realize higher economic gains. Furthermore, for countries currently regarded as being BSE (Bovine Spongiform Encephalopathy)- and foot-and-mouth-disease-free, it can constitute an important niche market. The present work is an adjunct to two previously reported studies[2,3] in which processes for converting bone to xenografts and powders for biomedical applications are described. Recently,[4] we have reported clinical studies in which the xenograft cores (excised from defatted/deproteinated bovine cancellous bone cubes) were shown to act as a successfully inte-

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grated osteoconductive scaffold in a sheep model. The procedures could be applied in any country where healthy livestock is slaughtered commercially and waste bone is plentiful.

Much of the current work in this paper is based on the study by Johnson *et al.*,[3] in which reprecipitated hydroxyapatite powders were produced by dissolving defatted bone directly in hydrochloric acid or nitric acid, centrifugation and simple filtering of the digest solution to remove insoluble matter, followed by addition of NaOH to the digest solution to reprecipitate the calcium phosphate. Problems arise in this preparation procedure due primarily to the presence of protein in the acid digest. Protein impurities give the digest solution a cloudiness that is difficult to remove. A second problem which is never mentioned in any publications dealing with processing of natural bone materials is discolouration imparted onto the HAp powders reprecipitated from usually orange-brown-coloured digests produced when nitric acid is used to digest the bone material. The origin of this colour change is exploited in protein chemistry where it forms the basis of the xanthoproteic test,[5,6] used to test for the presence of phenylalanine, tyrosine, and tryptophan residues in proteins or used as an analytical technique to probe the general level of protein in samples. In the xanthoproteic reaction, the nitric acid effectively nitrates the aromatic ring in the protein residues. An intensification of the orange colour of these nitroaromatic functional groups on the protein occurs in alkali solutions.

Despite the large number of studies involving bovine bone in biomedical applications, studies revolving around powders reprecipitated from defatted or sintered bone (with the powders being used for other applications such as for plasma spraying feedstocks) are practically nonexistent with only an earlier publication[3] written from our laboratory which deals with the issue. In that study, we had not sintered bone but were obliged to use hydrochloric acid rather than nitric acid as a digesting acid for dissolving defatted bone to avoid the strong discolouration in the resultant reprecipitated hydroxyapatite obtained after addition of hydroxide to the digest. Despite the use of hydrochloric acid, the presence of significant amounts of gelatine (from the bovine collagen) can be a serious nuisance impurity in such processing. Also, the use of hydrochloric acid as a digesting acid could lead to chlorapatites caused by partial substitution of Cl atom into the hydroxyapatite lattice.

To new entrants in this field, it is not obvious from the present literature as indicated by *Chemical Abstract* searches how important it is to remove the protein significantly from bone before attempting a digest to lead to the reprecipitated hydroxyapatite that can be later used in biomedical applications. Given the importance of hydroxyapatite in the biomedical research field and the large explosion of research into the use of this material, it is important to workers in this field or others to know about recommended procedures for its preparation from natural sources of calcium phosphate such as animal bone. The procedure described in this paper thus serves as an original contribution that spells out how to avoid the complication due to xanthoproteic relations which seriously discolour animal-derived powders and how cleaner, white powders can be obtained from such processing.

EXPERIMENTAL

Methodology for Preparing Reprecipitated Mammalian Bone Powder

Raw Bone Material. – Bovine femurs were sourced from cattle (breed unknown) slaughtered for human consumption in commercial abattoirs. Ovine bones were obtained as by-products of sheep processing in supermarket butchery. All bone was stored at –35 °C until the organics were removed, to prevent spoilage. Bovine femurs were sliced into 25 mm transverse slices using a water-cooled abrasive saw. The choice of saw was dictated by the results reported by Johnson *et al.* [2] who utilised a band saw for the slicing process and found that the heat generated by the blade caused the cut surfaces to seal making down stream processing more difficult. After slicing, the femurs were kept separate. Ovine bones were smaller than bovine bones and had a large amount of fatty material associated with them, hence cutting of the bones to smaller sizes was unnecessary. Defatting was achieved by boiling the bone slices in a domestic pressure cooker with tap water for six hours, with the water being changed every two hours. Sample weights were recorded before and after defatting to determine loss.

The pyrolysis step involved heating of all bone samples in a muffler furnace to *ca.* 1000 °C to ensure removal of the collagen plus other organics. The choice of this particular temperature is important as IR bands at 2200–2300 cm^{-1} due to protein-derived cyanates and cyanamides within the pyrolysed bone were detected when nominal temperatures < 1000 °C were used.[7] At the end of the pyrolysis, cancellous and cortical bone pieces were generally bleached white in colour and were very fragile. An overnight (*ca.* 12 hour) pyrolysis period was chosen to ensure removal of all organics. Both bone types retained their overall osseous architecture with the cortical bone exhibiting flaking of boney plates off its surface.

Additional separate processing experiments were also carried out for batches of bones during the boiling/defatting and pyrolysis stages to gain an idea of the approximate weight loss of the bone during each step.

Synthesis of Reprecipitated HAp. – Pyrolysed bone was crushed and then washed with deionised water (until the wash water pH was 7) followed by oven drying. One kilogram of pyrolysed bone was digested by adding to *ca.* 6.7 L of 20 % (vol. fraction) HNO_3 in a large beaker. The cloudy white solution was subsequently vacuum filtered into a custom-designed 10 L reactor vessel (see Figure 1).

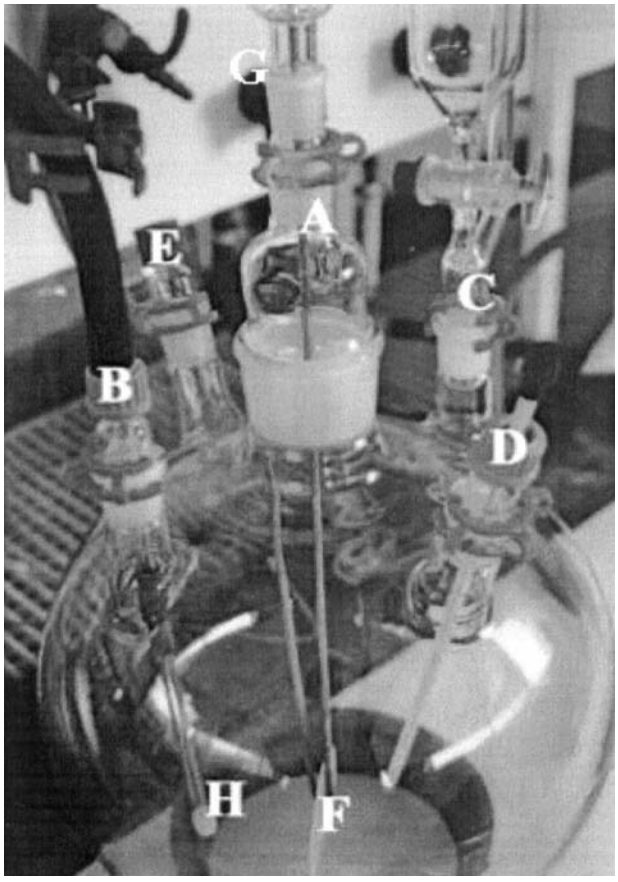

Figure 1. Photograph of the glass quickfit plus mechanical stirring apparatus used to produce kilogram quantities of HAp reprecipitated from nitric-acid-digested bone previously pyrolysed at 1000 °C.

The reactor vessel was a standard design which allowed the production of kilogram scale quantities of reprecipitated HAp from acid-digested mammalian bone. A large mechanically controlled stirrer bar (F) consisting of a stainless steel shaft and perspex blade was inserted through the large central neck (A) of the flask. Of the four smaller necks, two were vertical and two were sloping so that they faced the bottom of the flask. One of the vertical necks was used as a gas inlet (B) while the other was used for the addition of NaOH and as a gas venting outlet (C). The sloping necks were specifically designed to accommodate a thermometer (D) and pH probe (E) so that pH and temperature in the flask could be monitored during the course of the precipitation reaction. Warming of the flask was accomplished by using a water bath contained within a large stainless steel vessel heated underneath by a hotplate. Item G (not completely visible in Figure 1) on the flask set-up was an oil gland through which the stirrer rod shaft was rotated so that air (which contains CO_2) could not diffuse through the shaft gap and the lubricating gland into the HAp solid as carbonates.

After introduction into the reaction vessel, the filtered bone digest solutions were stirred and sparged (see item (H) in Figure 1) with nitrogen gas for two hours to purge out atmospheric CO_2. The solution was subsequently heated to 80 °C with the mechanical stirrer rate set to 240 rpm. Saturated NaOH solution (approximately 15 mol L^{-1}) was added slowly to the acidic digest which resulted in the formation of a heavy white precipitate. A solution of 7 mol L^{-1} NaOH solution was subsequently added at a rate of 200 mL min^{-1} until pH 9 was reached. Dropwise addition of NaOH was continued to keep the pH between 8.5 and 9.5. After the pH had stabilised the mixture was left for *ca.* 12 hours with heating. The precipitate was then aged for 48 hours without heating after which time the stirrer and the nitrogen gas stream were stopped and the precipitate allowed to settle for a further 24 hours. After decanting off the supernatant, the reaction vessel, which at this point, contained a white precipitate, was refilled with deionised water and swirled to wash the precipitate before being decanted off again. Washing was continued until no nitrate could be detected (by the brown ring test) in the washings. The hydroxyapatite solid slurry was subsequently transferred to a very fine monofilament filter cloth and filtered by squeezing through the fluid; this avoided problems of blockage and excessively long filtration times when using buchner funnels or sintered glass crucibles to separate off the solids. The solid was oven dried at 120 °C and the resultant white cake broken up into a fine powder by grinding.

The powders plus bone were characterised at various stages of their processing by a suite of spectroscopic and microscopic techniques. All X-ray diffractograms (XRD) were acquired using a Phillips PW1729 X-ray diffractometer with Cu-Kα radiation through a 0.2 mm slit with 2θ range of 10° to 70°. The tension was 35 kV and the current 30 mA. A speed of advance of 0.05° sec^{-1} and time constant of 1 s were set for each XRD. Malvern particle size distributions were performed on a Malvern Mastersizer S long bed with a MS17 sample introduction unit. The range lens was 300RF giving a size range of 0.05–900 μm. Beamlength was 2.4 mm and enough sample was added to produce an obscuration value between 10 and 20 %. The Malvern measurements of particle sizes and particle size distributions were compared in the presence and absence of ultrasonication of the suspended samples. Data analysis was carried out using a polydisperse model and assuming a HAp density[8] of 3.16 g cm^{-3}. All FTIR spectra of the ground bones and powders were recorded using KBr disks on a Digilab FTS-40 Fourier transform infrared spectrometer. Absorbance spectra were acquired between 4000 and 400 cm^{-1} at 4.0 cm^{-1} resolution over 16 scans. SEM micrographs were recorded at 20 kV on a S4000 Hitachi Field Emission Scanning Electron Microscope of samples mounted on double-sided carbon tape affixed to aluminium stubs. Elemental compositions of the bone powder were analysed by the commercial laboratories, Celentis Analytical and Hills Laboratories, Hamilton (New Zealand).The

TABLE I. Weight loss data for bovine bone that has been subjected to defatting and pyrolysis trials[a]

Bone type	Initial weight	Weight post-boiling	Weight loss on boiling	Weight post-pyrolysis	Overall weight loss
	g	g	%	g	%
Raw cancellous bovine bone	175.68	N/A[b]	N/A[b]	37.83	78.47
Defatted cancellous bovine bone	273.67	104.8	61.71	57.42	79.02
Raw cortical bovine bone	50.84	N/A[b]	N/A[b]	21.51	57.69
Defatted cortical bovine bone	98.29	56.98	42.03	40.61	58.68

[a]All weight data represent averages taken over 3 samples.
[b]N/A = not applicable.

elements, Fe, Cu, Mg, Na, Ni, Ca, Mn, Zn,, Cd, K, Co, Cr, Hg, Pb, Al, As, Li, Sb, Sr, Ti and ions NO_3^-, and PO_4^{3-} were analysed using a combination of inductively coupled optical emission spectrometry (ICP-OES), inductively coupled mass spectrometry (ICP-MS), molybdovanadate colorimetry (for PO_4^{3-} determination only), and (for NO_3^--nitrogen detection) flow injection analysis (FIA). Extraction of these elements from the powder samples involved in most cases HCl/HNO_3 digestion except for Pb, Cr and Ni which involved $HClO_4/HNO_3$ digestion. Nitrate ion was extracted with water from the powders, reduced to nitrite ion, and subsequently subjected to an FIA analysis.

RESULTS AND DISCUSSION

Processing Aspects and Characterisation of the Pyrolysed and Reprecipitated HAp Powders Prepared from Mammalian Bone

Defatting and Pyrolysis. – Table I is a summary of the weight loss data (for defatting and then pyrolysis at 1000 °C) for bones samples divided by species and type. Bovine cancellous bone lost on average 80 % total of its mass after processing whereas the denser bovine cortical bone lost 60 % total. Ovine bone lost between 75 and 90 % of its original weight after defatting and pyrolysis thus reflecting the high organic and fat content in the ovine bones obtained. In general, better results were obtained in the pyrolysis step if previously defatted bones were used rather than raw bones. Raw bones gave mixed results with some specimens exhibiting orange streaks in areas due most likely to Fe from blood stains. These colours did not show up in the reprecipitated powders. It was found that the optimum pyrolysis time for all bone types at 1000 °C was 3 hours. Cancellous bone from the condyle of bovine femurs took on average 2 hours to attain constant weight while bovine cortical bone took 3 hours to reach constant weight. Bones from ovine sources (not shown in Table I) took 2.5 hours to reach constant weight. These differences in pyrolysis time were due to differences in the composition of the bone material. Cancellous bone has a very open structure which allows the organic material to escape as gaseous pyrolysis products from the matrix more rapidly. Ovine bones were smaller and comprised more of the denser cortical bone which may have acted to retard the removal of the organic material by pyrolysis. For similar reasons, the cortical bone of the bovine femur, which was very dense and over 1 cm thick, did not also pyrolyse as rapidly as the cancellous bone matrix.

Most importantly, nitric acid digestion of the pyrolysed bone followed by reprecipitation in NaOH completely avoided the complications associated with the digestion process carried out using just defatted collagen-containing bone.[2,3] Digests were free of the orange-yellow colouration from xanthoproteic-associated reactions as were the heated reprecipitated slurries so that the end products were white powders suitable for biomedical purposes such as plasma spraying for instance.

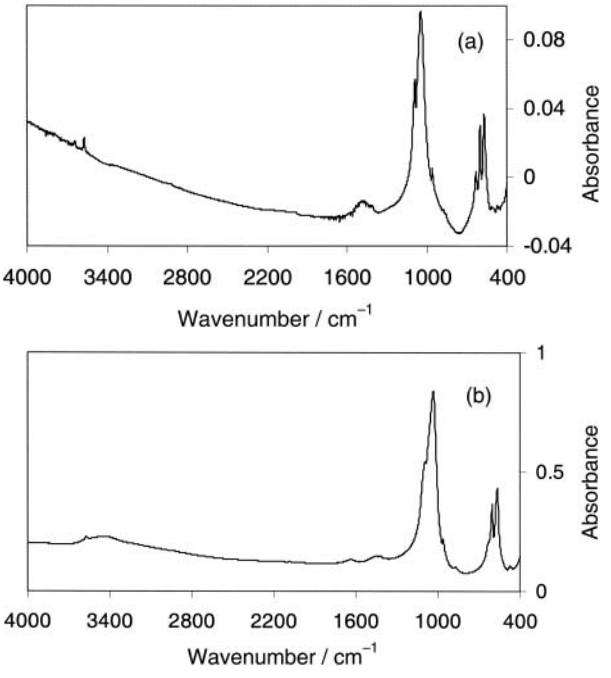

Figure 2. FTIR spectra of (a) bone after pyrolysis at 1000 °C and (b) HAp reprecipitated from acid-digested pyrolysed bone.

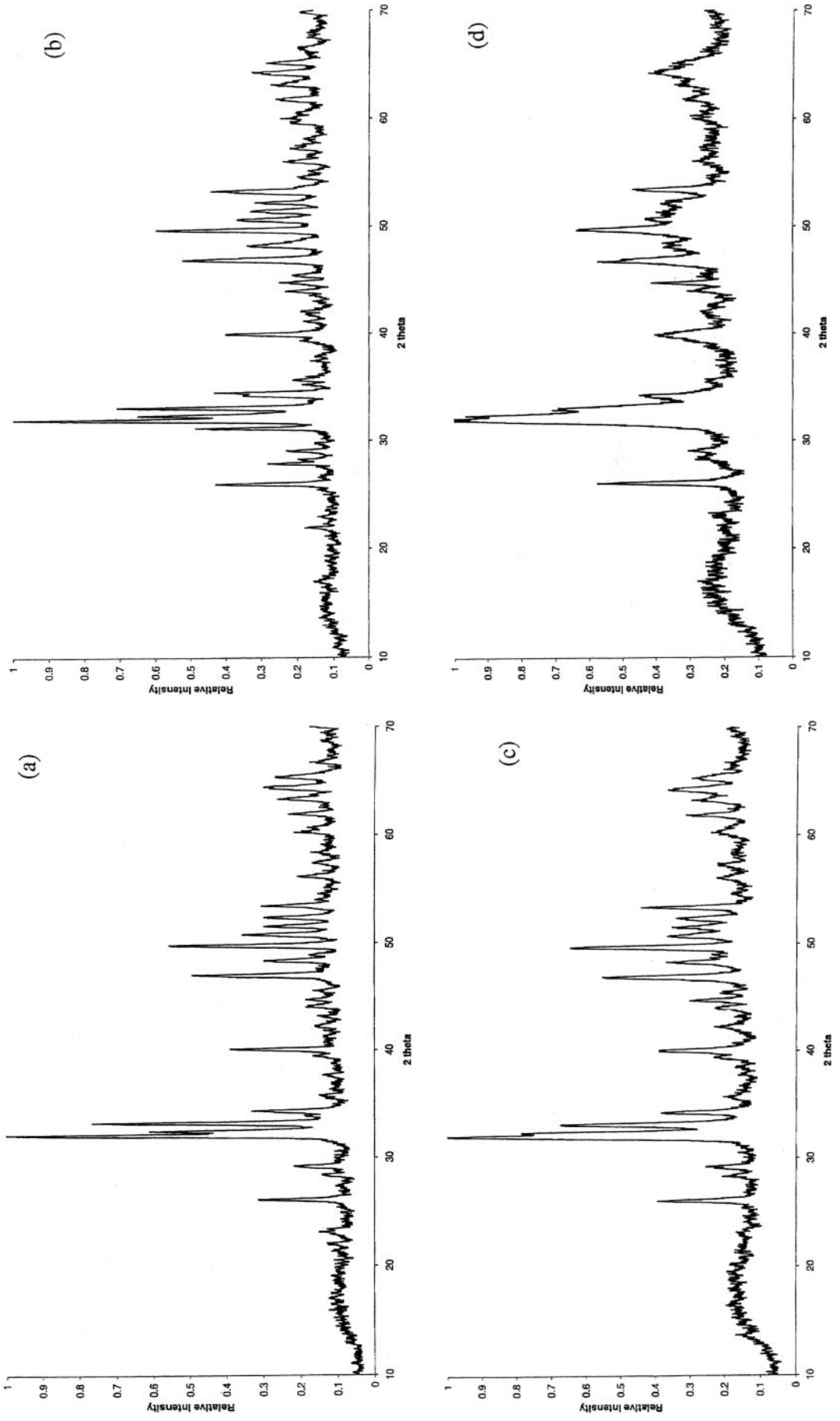

Figure 3. X-ray diffractograms of (a) bone after pyrolysis at 1000 °C, (b) Fluka »Tricalcium phosphate« corresponding to pure hydroxyapatite, (c) carbonated hydroxyapatite powder and (d) hydroxyapatite reprecipitated from acid-digested pyrolysed bone.

Characterisation of the Pyrolysed and Reprecipitated Bone

Spectral and X-ray characterisation of the pyrolysed bone and reprecipitated HAp were carried out to check the integrity of the calcium phosphate prepared by this procedure. FTIR and XRD characterisation was carried out of i) the pyrolysed bone and ii) the reprecipitated bone in order to assess morphology, phase purity, particle size distributions and the level of crystallinity. Figure 2a is an FTIR spectrum and Figure 3a XRD of bovine bone pyrolysed at 1000 °C. In general, the observed sharp intense features at 573, 602 and 1080 cm^{-1} are characteristic of crystalline HAp. Additional peaks at 870 (very weak) and *ca.* 1500 cm^{-1} were indicated, however some carbonate in the HAp structure. This was confirmed by an XRD of the pyrolysed bovine bone (Figure 3a, which, when compared with the XRD of pure Fluka brand hydroxyapatite (Figure 3b) and an XRD of a sample of known carbonated hydroxyapatite (Figure 3c), resembled more the XRD in Figure 3c than in Figure 3b. Carbonates would not be expected in the HAp lattice after pyrolysis at 1000 °C but may have re-entered the structure by ingress of atmospheric CO_2 into the bone during cooling of the bone pieces in air after removal from the muffler furnace.

In contrast, the FTIR (Figure 2b) and the XRD (Figure 3d) of the reprecipitated bone show expectedly broader peaks due to the lower crystallinity of the reprecipitated hydroxyapatite. SEM micrographs of the uncrushed pyrolysed bone (Figure 4a) showed that the osseous architecture and the hydroxyapatite crystal structure remained intact in bone pyrolysed to this temperature which is in agreement with work carried out by earlier workers in this field.[9] SEM micrographs of pyrolysed bone crushed by grinding (Figure 4b)show an irregular chunky or plate-like morphology for particles. This is distinctly different from SEMs of the reprecipitated HAp which show characteristic short rod-like shaped particles (see Figure 4c).

Malvern-Mastersizer-measured volume-based particle size distributions were acquired for the pyrolysed bone after grinding and compared with those for reprecipitated HAp powders. Two Malvern measurements were acquired for each sample, one for the sample without ultrasonication (not-US) and one for the sample with ultrasonication (US). Samples were compared to a commercial sample of Fluka hydroxyapatite. The results are summarised in Table II. In general, mechanically ground pyrolysed bone not subjected to any washing had large particle sizes as might be expected from such a coarse sample. Ultrasonication of these coarse particles had little effect on the overall particle size distributions. The average particle sizes for the mechanically ground bone materials would be expected to exhibit a strong dependence on the extent of mechanical grinding. For instance, the sample of ground pyrolysed bone subjected to washing (see Table II), had overall smaller particle sizes due either to the washing process itself which would have leached out CaO and or $CaCO_3$ from the sample or to the finer grinding of the sample. The washing was carried out because the presence of these components were thought to

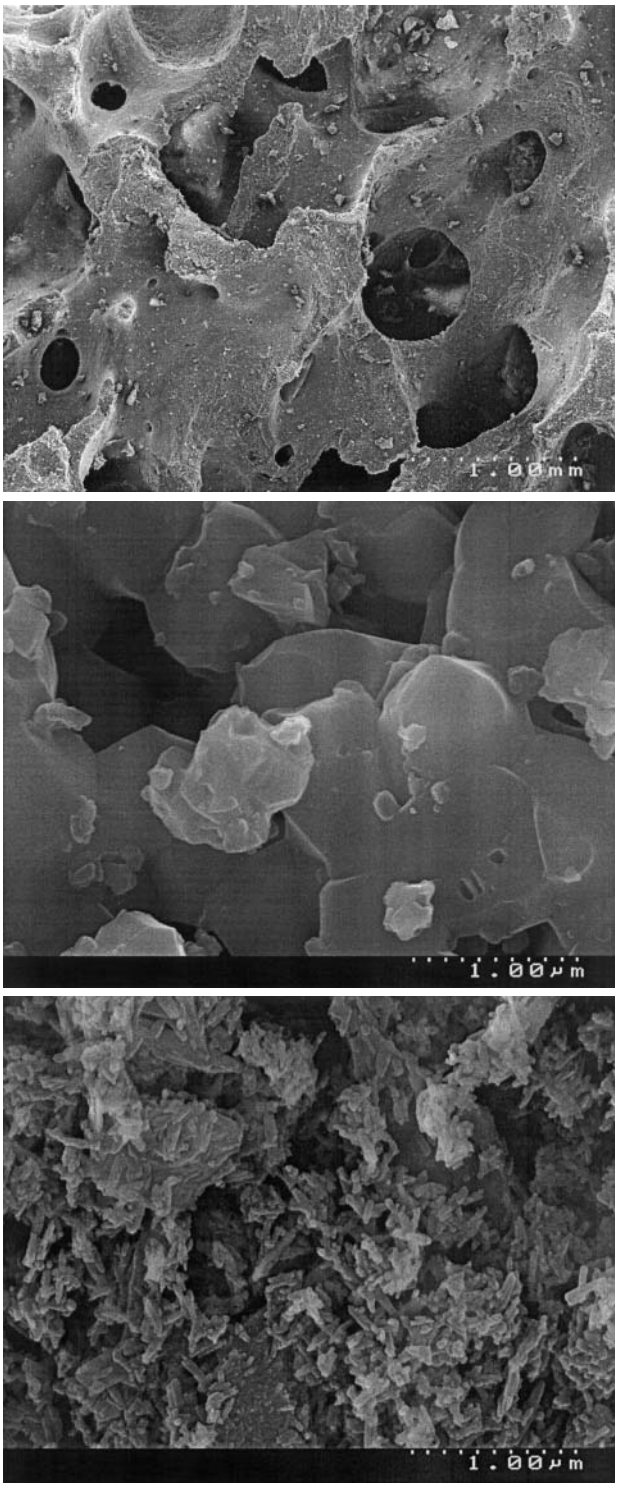

Figure 4. Scanning electron micrographs of (a) unground bone after pyrolysis at 1000 °C showing the retention of the osseous architecture, (b) pyrolysed bone crushed by grinding, (c) HAp powder reprecipitated from acid-digested pyrolysed bone.

TABLE II. Summary of Malvern Mastersizer results on pyrolysed and reprecipitated bovine bone powders

Description of powder	$D[4,3]^{(a)}$ / μm		Description of volume-based particle size distribution	
	Not-US[(b)]	US[(b)]	Not-US[(b)]	US[(b)]
Unwashed ground pyrolysed bovine bone pre-sieved to select particles > 75 μm	405	357	Particle sizes from 800 μm tailing to 0.1 μm	Similar shape and distribution to Not-US sample
Washed[(c)] ground pyrolysed bovine bone	22.3	9.7	Complex distribution from 0.1 μm to 500 μm	Similarly shaped distribution to Not-US sample but with particle sizes extending only to 100 μm
Acid digested and reprecipitated pyrolysed bovine bone, unsieved	64.7	23.9	Approx. unimodal distribution from 500 μm tailing to 0.1 μm	Approx. unimodal distribution from 100 μm tailing to 0.1 μm
Fluka BioChemika Microselect »tri-calcium phosphate« (*viz*., hydroxyapatite: $Ca_5HO_{13}P_3$)	519	14.9	Large aggregates > 1000 μm	Approx. trimodal distribution from 0.1 to 100 μm

[(a)]Average volume-based particle size computed by treating all particles as equivalent spheres.
[(b)]US = ultrasonicated during the Malvern data acquisition process, Not-US = not ultrasonicated.
[(c)]To remove $CaO/CaCO_3$ formed in samples during the pyrolysis and cooling process.

be responsible for a discovered pH increase measured in water samples containing the powders in suspension. The hydroxyapatite reprecipitated from acid digests of the pyrolysed bone generally possessed finer particles than observed with the ground pyrolysed bone powders. This is consistent with the morphological characteristics of the two powders as observed by SEM which shows that the reprecipitated powders possessed short rod like shapes whereas the ground pyrolysed bovine bone possessed more irregular morphology. In most cases in the Malvern measurements, ultrasonication during sample acquisition broke up particle aggregates in the recirculating water medium so giving smaller measured values for $D[4,3]$.

The elemental profiles as measured by ICP-OES, ICP-MS, FIA and molybdovanadate colorimetry of the pyrolysed bone HAp, washed pyrolysed bone HAp and reprecipitated HAp are shown in Table III. In general, levels of the elements, Cd, Hg, Pb, As and Sb in the samples are either very low or below the detection limits. For pyrolysed bone, the levels of the elements analysed are generally consistent across different samples. The Sr levels (190–400 ppm) also appear consistent with what has been measured previously in this laboratory for bone in various states. Some of the observed differences in elemental levels across differently processed samples (*i.e*. from going from pyrolysed bone to pyrolysed washed bone to bone reprecipitated from pyrolysed bone) are readily understood in terms of changes brought about by the processing steps. For example the differences in Ca : P mole ratio between pyrolysed bone (1.91) and the other two samples (1.74, 1.75) is caused by the washing away of the $CaO/CaCO_3$ produced by pyrolysis and cooling or by the act of digestion and reprecipitation of the solid which would see any excess Ca go into solution. The large difference in nitrate nitrogen levels between the

TABLE III. Elemental profiles of bone after pyrolysis at 1000 °C and HAp powders reprecipitated from acid-digested pyrolysed bone

Element	Pyrolysed bone	Pyrolysed washed bone	Reprecipitated pyrolysed bone
Ca/%	44	40	40
P/%	17.8	17.46	17.64
Ca : P mole ratio	1.91	1.74	1.75
Mg/%	0.67	0.64	0.65
Na/%	1	1.81	1.61
K/ppm	570	260	<30
Fe/ppm	107	97	172
Cu/ppm	9.9	24.8	5.1
Mn/ppm	1	2	3
Zn/ppm	28	55	42
Al/ppm	27	99	137
As/ppm	0.7	0.9	1.3
Cd/ppm	<0.03	0.09	0.06
Cr/ppm	1	7	1
Hg/ppm	<0.05	<0.05	<0.05
Ni/ppm	<1	<1	2
Pb/ppm	1.2	6.3	2.8
Li/ppm	15.1	22.3	8.2
NO_3-N nitrate nitrogen/ppm	1	1	120
Sb/ppm	1.6	1	2.1
Sr/ppm	384	352	192

washed/unwashed pyrolysed bone samples and the reprecipitated bone samples is a direct result of using nitric acid for the digestion. It is obvious that despite extensive washing and filtration a low level of nitrate ion remains in the solid.

K and Sr are readily removable elements from the pyrolysed bone given the levels of these elements decrease upon washing and further upon reprecipitation. Mg levels are remarkably consistent over samples showing it is intimately associated with the hydroxyapatite lattice and hence not easily removed. Some of the elements analysed for demonstrate some inconsistent trends with processing. The main point of importance from these elemental analyses is that the heavy metal levels such as Cd, Hg and Pb in these processed bone materials are very low hence favouring their use in biomedical applications.

The most likely use of these bone-derived powder materials is as a plasma spray feedstock and this application of the powder to make plasma spray coatings on titanium has already been discussed in an earlier publication[3] and is the subject of current and future research. Another application of the bone powders either prior to or post reprecipitation processes is as a controlled release material for bone morphogenetic proteins, a topic of increasing interest in the biomaterials research community.[10]

Considerations for Creating an Industrial Scale Manufacturing Process for Reprecipitated Hydroxyapatite from Bone

In order for such a bone sintering/digestion/reprecipitation process to be extended to an industrial scale, many factors would need to be considered all of which will determine the final cost of the biomedical material on the market. It is difficult to give actual figures of cost because this will depend on where the process is carried out around the world and how it is carried out industrially. It would be important, however, to maintain standards of production within international levels relating to hygiene and quality control. An important factor in the production of this material would be to guarantee that the animal bone being manufactured was completely free of any prion material. Sourcing the material from a country which is known internationally to be BSE-free would be a distinct advantage but to satisfy international safety standards, protocols such as extensive heating such as would be achieved *via* sintering at 800–1000 °C to remove all organic matter leaving a mineral shell followed by dissolution in nitric acid and reprecipitation with alkali to reprecipitate the hydroxyapatite would guarantee a material that was completely free of prions. Alkali is well known to deactivate prions.[11] By virtue of this reconstitution as a synthetic inorganic material, it is possible that the laws governing the classification of the material in terms of a material obtained from animal sources could be relaxed or reinterpreted because of the complete transformation of the material into a synthetic compound completely different from the original material. Such issues can be complex and will depend very much on the country in which the processing is chosen to take place.

Other industrial processing stages of this material which may attract costs would be the electricity costs for pyrolysing the bone to high temperatures, chimney stack filters to remove volatiles given off by the bone materials in order that environmental compliance laws are satisfied, and the need to wash away nitrate from the product using water which may subsequently require remediation by removal of the nitrate and the recycling of the water back into the plant for use in washing more product. Nitrate could also be recycled back to nitric acid and released so that it is not released to the environment where it would cause eutrophication of natural waterways.

One major determinant of the final cost of manufacturing could depend on whether the choice was made to attach the process to an existing large scale manufacturing process (by making minor adjustments so minimising the costs) or else create only a small scale pilot-sized plant that had relatively small kilogram outputs to supply a small niche market. Overall costs could also well be lower across the board if production was carried out in countries in which raw material or labour was far cheaper than in more developed countries or in countries (with currencies pegged to the United States (US) dollar) that have low values compared to the US dollar. If countries are remote from major trading partners then the costs of transportation could be a significant counterbalance to such advantages so placing emphasis on the need to have a lower exchange rate for market competitiveness and could favour options such as supplying of niche markets in closer geographical locations or local markets within the manufacturing country.

CONCLUSIONS

A method for avoiding the discolouration problems encountered in the production of reprecipitated HAp powders for biomedical purposes from mammalian bone has been reported. Pyrolysis to remove the protein cause of the decolouration prior to digestion and reprecipitation is key to this method. Washing and reprecipitation are necessary for producing fine powders of hydroxyapatite and to remove CaO produced in the bone during pyrolysis. The levels of heavy metals such Hg, Pb, Cd and Sb are either low or undetectable in the powders produced so making them suitable in biomedical applications. Some considerations of the factors that may contribute to costs of expansion of this process into industrial scale were discussed. In general, it has been recognised in this research that this method is a potentially viable method for producing biomedical quality HAp from plentiful bone

stocks in countries remote from major producers of synthetic HAp where the development of this resource could lead to greater utilisation of a waste resource and the development of niche markets.

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SAŽETAK

Metoda pomoću koje se zaobilazi ksantoprotetički praćeno gubljenje boje u pretaloženome hidroksiapatitu priređenome iz kostiju sisavaca

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Razvijen je postupak za dobivanje kilogramskih količina biomedicinski uporabivoga pretaloženoga hidroksiapatitnoga praška iz kostiju sisavaca. Postupak se odvija na sljedeći način. Najprije se kuhanjem uklone masti s kostiju, a zatim se pirolizom na 1000 °C uklone kolagenski proteini i dobiju su izbjeljene kosti. Ove se kosti smrve i otope u dušičnoj kiselini. U dobivenoj otopini nema žutoga obojenja koje izaziva mrlje što je uobičajena pojava kod otapanja kosti dušičnom kiselinom. Ako se toj otopini doda NaOH u dušikovoj atmosferi i uz grijanje na 70 °C i miješanje dopusti zrenje taloga dolazi do taloženja hidroksiapatita. Tako dobiveni produkt je karakteriziran pomoću spektroskopskih i mikroskopskih tehnika te tehnika određivanja veličine čestica. Ove su analize pokazale da je dobiveni prašak niskokristaliničan hidroksiapatit konzistentne morfologije, koji ima ili prihvatljivo male vrijednosti teških metala ili vrijednosti ispod granice određivanja. Stoga predložena metoda daje potencijalno vrijedan prašak za biomedicinske primjene kao npr. za izradu proteza i sl.