A simple route to full structural analysis of biophosphates and their application to materials discovery†

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An integrated suite of synthesis and characterisation techniques that includes synchrotron-based single crystal, powder X-ray diffraction, nuclear magnetic resonance and electron diffraction have been employed to uncover two new distinct structures in the Ca,Ba2−xP2O7 polymorphic phosphate system. These materials have particular relevance for their application as both biomaterials and phosphors. Calcium barium pyrophosphate, CaBaP2O7, was shown by a combination of spectroscopic and diffraction techniques to have two polymorphs distinct in structure from all of the five previously reported polymorphs of Ca, Sr and Ba pyrophosphate. A high temperature polymorph HT-CaBaP2O7 prepared at 1200 °C is orthorhombic, of space group P212121 with a = 13.0494 Å, b = 8.9677 Å, c = 5.5444 Å. A low temperature polymorph LT-CaBaP2O7, prepared below 1000 °C, is monoclinic with space group P21/c and dimensions a = 12.065 Å, b = 10.582 Å, c = 9.515 Å, β = 94.609°.

Introduction

Phosphates and their related salts are ubiquitous within modern society, with strong demand in particular from the agriculture and health sectors.1–7 These applications, along with emerging uses in electrochemistry,8–10 have created a global shortage of phosphates. Because of the many bonding modes of the PO4 tetrahedron,11–13 most phosphates develop complex intermixtures in biological settings,14,15 or exhibit intricate polymorphism with metal salts.16–18 These twin challenges of supply and purity mean that efficient synthesis of pure materials and their ready characterisation is an important and pressing goal. The consequences of phase impurity and/or its lack of detection can be severe. For example, the presence of small impurities of calcium phosphates within hydroxyapatite coated bone implants can create a cytotoxic surface, severely limiting the ability of the implant to graft successfully.17 The loss of calcium phosphate from the bones is associated with osteoporosis and its accumulation in joints can lead to gout.19,20

Many of these polymorphs form at lower temperatures and in small quantities, rendering a sample that has small amounts of amorphous material present. This can be difficult to detect with traditional methods such as powder diffraction.21–28

Calcium pyrophosphate has at least three polymorphs, α, β, and γ polymorphs of the anhydrous compound, and a further three polymorphs of the hydrated form.29,30 Sr2P2O7 is known to be polymorphic. The low-temperature β-phase is tetragonal31 and probably isostructural with β-Ca2P2O7.32 The structure of the high-temperature α-phase has been determined first in the non-centrosymmetric Pna21 space group,33 subsequently revised to the centrosymmetric Pmna space group.34,35 This space group was later confirmed and the structure refined.36 Barium pyrophosphate crystallises in a quite different hexagonal form, σ-Ba2P2O7, when prepared by solid state reactions of barium carbonate with ammonium hydrogen phosphate.37 An α-form of barium pyrophosphate isostructural with β-Ca2P2O7 has been prepared using hydrothermal techniques or from a suitable flux.38 X-ray determination of the σ-Ba2P2O7 structure indicated disorder in the bridging oxygen atoms of its P2O7 groups.39 A large number of mixed pyrophosphates XYP2O7 (X = Ca, Sr, or Ba; and Y = Ni, Mn, Zn, etc.) have been investigated by various workers.15,39–41 Many have the monoclinic α-Ca2P2O7 structure while a number of the barium compounds are triclinic.37,42–45

Herein we illustrate the successful usage of a suite of novel synthetic approaches and alternative characterisation techniques that deliver high purity samples, ready detection of phase impurities even at low levels, and more complete structural characterisation. We have applied these approaches to the (Ca,Ba2−x)P2O7 system...
biophosphate system and discovered two new heterobimetallic structures, and fully resolved the atomic structure by combining complementary characterisation methods.

**Experimental**

**Sample preparation**

**LT-Ca$_2$P$_2$O$_7$.** CaHPO$_4$ (BDH, 1.97 g, $1.45 \times 10^{-2}$ mol) was heated at 350 °C under vacuum ($5 \times 10^{-3}$ Torr) for 4 days. Reduced pressure was employed to allow for higher temperatures to be reached for improving crystallisation in the absence of protons, believed to initiate phase transitions to higher temperature polymorphs. 1.83 g (99.5%) of Ca$_2$P$_2$O$_7$ material was recovered. X-ray diffraction (XRD) showed that the desired product had been formed. However diffusivity was observed during electron diffraction experiments. A further sample of CaHPO$_4$ (BDH) was taken and pressed at 8 tons pressure for 60 seconds to form a pressed pellet. This sample was then heated under vacuum ($5 \times 10^{-3}$ Torr) to 400 °C for 6 days to give a better crystallised material.

**HT-Ca$_2$P$_2$O$_7$.** 2.00 g CaHPO$_4$ (BDH, 2.00 g, $1.47 \times 10^{-2}$ mol) was pressed into a pellet at 8 tons pressure for 60 seconds and heated at 600 °C for 24 hours. This yielded 1.82 g of white powder (98.5%), which exhibited β-Ca$_2$P$_2$O$_7$ components in XRD powder patterns.

**σ-Ba$_2$P$_2$O$_7$.** BaHPO$_4$ (3.00 g, $1.29 \times 10^{-2}$ mol) was heated at 990 °C for 11 days and 2.85 g of white powder was recovered.

**CaBaP$_2$O$_7$.** Stoichiometric amounts of CaCO$_3$, BaCO$_3$ and $\text{NH}_4\text{H}_2\text{PO}_4$ were reacted together successively at 400, 800, 1000 and 1200 °C with intermediated grinding and pelleting. LT-CaBaP$_2$O$_7$ was afforded in quantitative yield after calcination at 1000 °C, and HT-CaBaP$_2$O$_7$ was prepared, also in quantitative yield with calcinations at 1200 °C.

**Characterisation**

Transmission electron microscopy (TEM) was conducted on a JEOL JEM-2011 microscope operated at 200 kV. The electron diffraction patterns were recorded on both a Gatan UltraScan CCD camera and electron-sensitive film. X-ray diffraction was performed on the powder diffraction beamline at the Australian Synchrotron with X-ray energy of 15 keV. Each sample was placed in a 0.3 mm diameter quartz capillary and examined over the range of $2^\circ < 2\theta < 82^\circ$. Rietveld refinements were subsequently performed using Topas™ V4.2. The $^{31}$P NMR spectra were obtained by the using a Bruker Avance 400 NMR spectrometer. Single crystal X-ray crystallography was performed on the microcrystal (MX2) beamline of the Australian Synchrotron at a wavelength of 0.71019 Å using the Blu-Ice46 interface for data collection and the XDS47 software package for initial processing (full details in ESI†). CL spectra were obtained on a Gatan Mono CL3 attached to a FEI Quanta 200 SEM with a Hamamatsu R943-02 detector. During a typical measurement, the area analysed was $14.86 \times 12.71 \mu m$. The samples were cooled to 77 K before analysis in order to provide a stronger response.

**Results and discussion**

Solid state $^{31}$P NMR is a particularly sensitive technique for characterising polymorphism within phosphates. As shown in Fig. 2, both the peak position and lineshape are strong indicators for phase identification. For LT-Ca$_2$P$_2$O$_7$ and HT-Ca$_2$P$_2$O$_7$, there is a clear upfield shift and change from four environments to two. The $^{31}$P NMR results show a clear difference in the phases of these two materials, which warranted further investigation. Our solid state synthesis protocols yielded single crystals of LT-CaBaP$_2$O$_7$ of suitable size for examination with synchrotron radiation. As...
shown in Fig. 3, LT-CaBaP$_2$O$_7$ crystallises in the monoclinic space group $P2_1/c$ with dimensions $a$ 12.065 Å, $b$ 10.582 Å, $c$ 9.515 Å, $\beta$ 94.609°. The structure contains four P-sites in its asymmetric unit of which three have quite similar environments, different to the fourth phosphorus. This leads to the observed NMR pattern of two peaks in a 3:1 ratio. In this instance no site swapping between calcium and barium atoms was observed; there are preferential calcium and barium sites. This has implications for ferroelectric materials where this kind of order is important for inducing anisotropy. Whilst our solid state crystallisation methods were successful in delivering single crystals of LT-CaBaP$_2$O$_7$, the crystals derived from HT-CaBaP$_2$O$_7$ were too small, even for analysis with synchrotron radiation. Nevertheless, it was possible to obtain a full structural refinement on synchrotron-based powder diffraction collected for the high temperature phase, using a simulated annealing algorithm with rigid body constraints for the P$_2$O$_7^{4-}$ anion (see Fig. 2 and 3). Details of the experimental conditions and of the structure solution are given in Table 1 of the ESI. Refined atomic coordinates, occupancy and thermal parameters for the HT-CaBaP$_2$O$_7$ phase are reported in Table 2. The refined structure is again confirmed by $^{31}$P solid state NMR and electron diffraction techniques employed to complement the X-ray data. LT-CaBaP$_2$O$_7$ crystallises in orthorhombic space group $P2_12_12_1$ with $a$ 13.0494 Å, $b$ 8.9677 Å, $c$ 5.5444 Å, $V$ 648.82 Å$^3$. In this case there is clear intermixing between the metal sites as evidenced by the slight variations in peak intensity data during the Rietveld refinement (Fig. 3). Presumably this intermixing occurs due to the higher temperatures employed providing activation to solid state diffusion. The refined structure indicated the presence of two chemically distinct phosphorus sites in equal ratios, which the solid state NMR confirms. Furthermore, the increased broadening in the NMR can be attributed to the Ca–Ba site intermixing.

Electron diffraction analysis (Fig. 4) confirmed both the single crystal and powder diffraction structures, with the same crystal parameters being readily indexed. This is a powerful technique for the assignment of structures to predominantly microcrystalline materials such as the present phosphates, as an electron beam as thin as 1 nm can be used for probing nanoscale domains. Structured diffuse scattering was observed in the selected-area electron diffraction patterns of all samples, but not in the corresponding high-resolution TEM images; this is indicative of the dynamic nature of the bridging P–O–P group in this system.

Luminescence provides further information regarding the bulk properties of the family of materials. Luminescence of pyrophosphates has long been investigated and changes in luminescence with structure have been remarked on and attempts have been made to correlate these changes with the atom environments in the phosphates. Most recently, remarkable differences were observed in the luminescent properties of long lasting phosphors based on the three polymorphs of zinc phosphate. As shown in Fig. 5, inclusion of calcium atoms into the barium pyrophosphate structure leads to smaller bandgap emissions, which are more marked for the LT-CaBaP$_2$O$_7$ in comparison to its high temperature counterpart. These results demonstrate that both the elemental compositions as well as the polymorphic structure may be controlled to deliver control of light-emissions within these well utilised phosphors.

Conclusions

Calcium barium pyrophosphate, CaBaP$_2$O$_7$, is dimorphic. Its formation by solid state reaction was monitored by $^{31}$P solid-
Fig. 4 (a) Selected-area electron-diffraction pattern of the [001] zone axis of HT-CaBaP$_2$O$_7$, yielding $d_{100} = 13.05$ Å and $d_{010} = 8.94$ Å; (b) convergent-beam electron-diffraction pattern along the [001] zone axis of HT-CaBaP$_2$O$_7$ showing Gjønnes–Moodie (GM) lines on odd reflections along both principal axes in accordance with space group P2$_1$/c; (c) selected area electron diffraction pattern of the [100] zone axis of the LT-CaBaP$_2$O$_7$ showing $d_{010} = 10.57$ Å, and $d_{100} = 9.53$ Å. Note the weak 0k0 and 00l reflections for $k = 2n$ and $l = 2n$, respectively. In convergent beam mode GM lines were observed for these reflections in accordance with space group P2$_1$/c; (d) selected area electron diffraction pattern of the [001] zone axis of LT-CaBaP$_2$O$_7$ showing weak intensity for $k = 2n$. In convergent beam mode GM lines are predicted on the 0k0 reflections for these odd reflections in space group P2$_1$/c (horizontal axis, $a = 12.08$ Å, and vertical axis, $b = 10.57$ Å).

structure determination using X-ray diffraction in a synchrotron beam.

A micron sized single crystal of the lower temperature polymorph was obtained from a crushed sinter but this proved to be of sufficient size for structure solution in the synchrotron beam.

The structure of the high temperature form was solved from the powder diffraction pattern obtained in the synchrotron. It is perhaps of interest to note that the unit cell dimensions of this form led us to initially assume it was isostructural with one of the forms already reported for Ca, Sr and Ba pyrophosphate. The quality of the data obtained from a synchrotron and the use of Rietveld refinement allowed us to obtain the correct structure.

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