

Synthesis of peroxyapatite by hydrothermal processing

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Abstract. Peroxide ions in apatite provides an additional resource for imparting an antibacterial capability in apatite. A hydrothermal process has been developed for including peroxide ions into the apatite lattice. Three oxygen generating compounds (hydrogen peroxide, ammonium persulphate and peracetic acid) were investigated for generating peroxyapatite. Hydrogen peroxide provides the highest peroxide containing apatite. Both the oxygen generation and crystallization of apatite represented the two critical factors for producing peroxyapatite. Unlike with high temperature processing, the cooling rate did not influence the retained peroxide content. This new process provides a building block for investigating antibacterial properties of peroxyapatite produced by a low temperature process.

Introduction

The incidence of infection can have devastating effects for the longevity of implants; implants should be designed to minimize these undesirable events. Hydrogen peroxide is commonly used on skin abrasions and is very effective, but high concentrations for internal use may damage cells involved in bone remodelling. Low peroxy concentration and slow release provides a new avenue for imparting additional antibacterial activity. This work will investigate a new process for producing peroxyapatite using a hydrothermal process.

The body is already equipped to use oxidative species for defence and has compensating effects to ensure that these species stay within allowable limits. Phagocytes use peroxy ions to combat bacteria [1], and osteoclasts use reactive species to assist resorption [2]. Enzymes keep oxidative species at allowable levels [3]. Peroxide is thus a chemical species familiar by the body, and could be included in biomaterials.

Inclusion of peroxy ions inside the structure of biomaterials is possible only with a select few biomaterials. Apatites offer the replacement of hydroxyl ions within the column with oxygenated species; the less densely occupied hydroxyl column not only provides the possibility of substitution, but also accepts larger linear molecules or charged oxygen species neutralized by smaller cations [4]. Rey studied oxygenated apatites and suggested the possibility to replace OH⁻ ions with neutral molecules such as oxygen O₂ and hydrogen peroxide H₂O₂ or a variety of charged oxygen species like the superoxide ion O₂⁻, the peroxide ion O₂²⁻ or polyoxide species O₃²⁻ and O₄²⁻ [5]. Despite this flexibility of substitution, the maximum concentration is restricted to two charged species within each apatite unit cell. There are no studies in the literature to suggest whether this concentration is sufficient for combating bacteria.

Three approaches have been reported for including peroxide in apatite. These include synthesis by 1) precipitation of apatite in aqueous solutions containing H₂O₂ [6, 7], 2) hydrolysis of beta tricalcium phosphate in H₂O₂, 3) heating apatites at high temperatures followed by exposure to oxygen [8, 9]. Hydrolysis of beta tricalcium phosphate has achieved a 40% replacement of hydroxyl ions. The highest replacement, between 68% and 80%, occurs by heating in oxygen at 900 °C or

higher. The hydrothermal approach has not been investigated and may offer an alternative to the above mentioned approaches.

The objective of this work is to examine whether peroxyapatite can be produced in a hydrothermal process with the use of three oxygen generating compounds.

Materials and Methods

Synthesis of reactants. An amorphous phase was synthesized by mixing a strontium nitrate solution into an ammonium hydrogen phosphate and an ammonium carbonate containing solution. The suspension was mixed for 10 minutes, filtered, rinsed with deionized water, filtered and then treated with ethanol before oven drying. Sr apatites accommodate higher concentrations of oxygen, and so were chosen instead of calcium apatites [7].

Hydrothermal processing. Powder was placed in a hydrothermal bomb and heated for different time periods. The amorphous calcium phosphate was accompanied by three oxygen generating species: H_2O_2 , $(\text{NH}_4)_2\text{SO}_4$, CH_3COOOH . The temperature for 30% hydrogen peroxide, ammonium sulphate and peracetic acid was 150 °C, 120 °C and 80 °C, respectively. The bomb was placed in a preheated oven and after heating cooled under running water.

Characterization and analysis. Powder was analyzed with X-ray diffraction to confirm the apatite phase after hydrothermal treatment. X-ray diffraction patterns were obtained on a Bruker D8 ADVANCE diffractometer, recorded from 5° to 60° using Cu $\text{K}\alpha$ radiation ($\lambda = 1.54180\text{\AA}$ generated at 40 mA and 40 kV) at a 0.2° step size.

Diffuse reflectance spectra in the UV to visible range were obtained with a PerkinElmer Lambda 950 spectrometer from 190 nm to 1100 nm with a resolution of 0.05 nm.

A classical redox titration aided peroxide quantification in apatite. Permanganometric titration involved an oxalic acid potassium permanganate KMnO_4 solution (0.005 M) in acidic media. A change in colour of permanganate ions indicated the end point of titration.

In the reaction: $\text{MnO}_4^- + 8\text{H}^+ + 5\text{e}^- \rightarrow \text{Mn}^{2+} + 4\text{H}_2\text{O}$ the standard potential is $E^\circ = +1.51\text{ V}$. During titration, the peroxide analyte is reduced: $\text{H}_2\text{O}_2 \rightarrow \text{O}_2 + 2\text{H}^+ + 2\text{e}^-$

The apatite powder was dissolved in concentrated perchloric acid and then diluted with deionized water before dropwise addition of the 0.005 M KMnO_4 solution. The titration endpoint was reached when the purple color of the permanganate ion was stabilized.

Results and Discussions

Different oxygen generation compounds were considered for hydrothermal treatment. The simplest was H_2O_2 , that upon heating produces water and oxygen gas; with greater decomposition at higher temperatures. Ammonium persulphate required a lower temperature to generate oxygen, but forms both oxygen and sulfur trioxide when dissolved in water. As a solid, it decomposed at 160 °C to form pyrosulphate and a 3.5% yield of oxygen [10]. Peracetic acid requires the lowest temperature and produces acetic acid and oxygen with a 10% yield. The highest concentration of oxygen was produced by hydrogen peroxide, at a yield of 47%.

Crystallization to apatite increased with processing temperature, Fig 1. At 80 °C when treated with peracetic acid, nanosized calcium phosphate crystals formed, as seen by a broad peak corresponding to the position for the most intense apatite peak. At 120 °C, the powder was more crystalline. At 150 °C, a higher crystallinity apatite formed. This highlighted the importance of temperature in adjusting the crystallinity. Only temperatures above 120 °C were suitable for the 3 hour synthesis period.

Generation of peroxyapatite depended on a) the temperature of oxygen generation and b) a sufficiently high temperature for crystallization. Heating with peracetic acid at 80 °C was sufficient to produce oxygen, but insufficient for crystallization to apatite. For ammonium persulphate, less oxygen was produced but greater crystallization was noted. Processing at 150 °C with H_2O_2 - the cleanest oxygen generator - produced the highest crystallinity.

Peroxide inclusion in the apatite lattice introduces a change in colour that could be followed by UV-Vis spectroscopy [11]. Yellowing of apatite corresponded to a peak in the UV-Vis spectra at 270 nm, Fig 1. Hydrogen peroxide treated powder led to a clear peak, indicating successful generation of oxygen and inclusion in the apatite structure. About 12% peroxide was included in apatite from the ammonium persulphate treatment. A colour change was not seen by the naked eye or registered by UV-Vis spectroscopy for peracetic acid treated powder. Titration with permanganate ions confirmed peroxide and provided a correlation with the 270 nm UV-Vis peak; equating to a 23% peroxide inclusion in apatite from the use of 30% H_2O_2 .

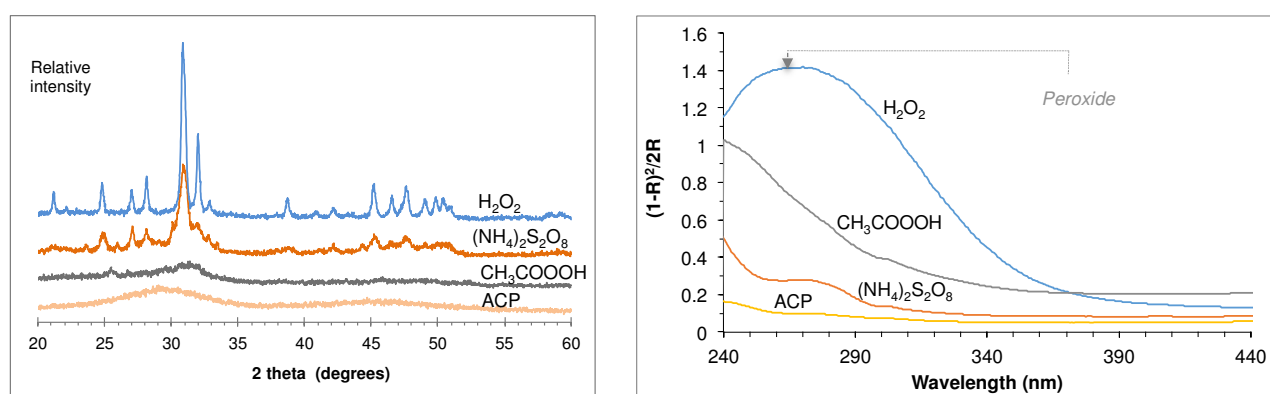


Figure 1. The X-ray diffraction pattern and UV-Vis spectra of calcium phosphate powder after treatment with peracetic acid CH_3COOOH , ammonium persulphate $(\text{NH}_4)_2\text{SO}_4$ and hydrogen peroxide H_2O_2 , showing the best formation of peroxyapatite with H_2O_2 .

High temperature treatment above 1200 °C has shown peroxide ion instability, requiring rapid cooling to retain peroxyapatite, and so the effect of cooling rate was investigated in hydrothermally treated powder. The hydrothermal bomb was removed from the furnace and cooled under running cold water, or left to cool without any cooling medium. UV-Vis spectroscopy did not show any change with the cooling rate for the H_2O_2 treated powder, Fig 2. Peroxide remains stable regardless of the cooling conditions in hydrothermal processing, unlike in high temperature processed peroxyapatite.

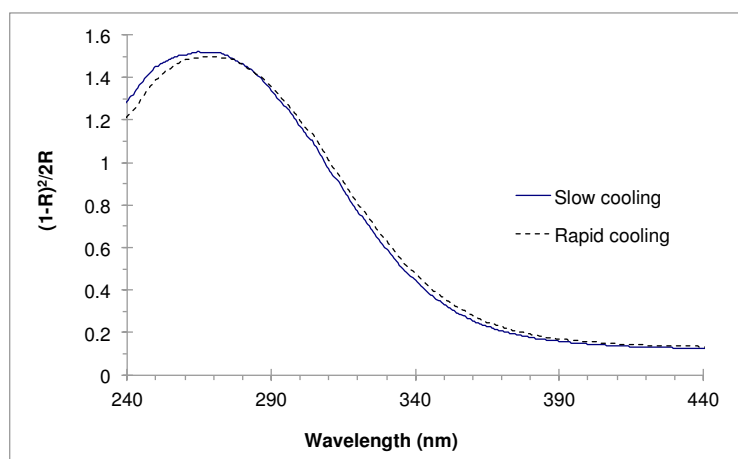


Figure 2. The UV-Vis spectra of peroxyapatite processed under rapid cooling and slow cooling conditions shows that cooling rate has no effect on the retained peroxide content.

The amount of available oxygen only slightly influenced the amount of peroxide incorporated in apatite. Greater quantities of powder resulted in lower amounts of incorporated peroxide, as seen by the less intense peaks in the UV-Vis spectra, Fig 3. UV-Vis peak heights of 1.3, 1.4 and 1.5 corresponded to 22%, 23% and 24% peroxide incorporation. Additional means need to be addressed to further increase the concentration of peroxide ions.

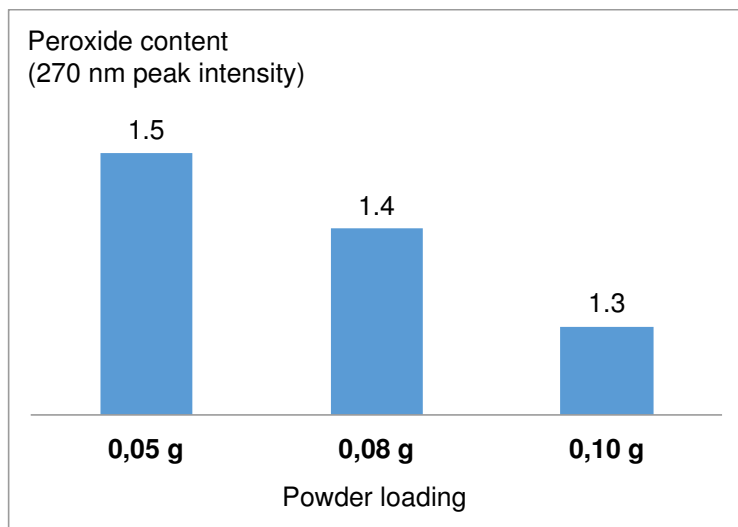


Figure 3. The peroxide content in peroxyapatite made with H_2O_2 , as per the yellow colour intensity detected in the 270 nm UV-Vis peak.

Hydrothermal synthesis shows another technique means for producing peroxyapatite. Processing at 150 °C was at a significantly lower temperature than the high temperature process, above 1000 °C [11]. This new process is simpler, faster and does not require rapid cooling conditions, like in high temperature processing. More concentrated hydrogen peroxide solutions are expected to incorporate more peroxide in the apatite lattice. Present work is addressing the use of 50% H_2O_2 as an alternative to 30% H_2O_2 solutions.

Further work will address supplementary approaches for further increasing peroxide inclusion from hydrogen peroxide, and determine the flexibility of the processing conditions for producing peroxyapatite.

Summary

Hydrothermal treatment has been applied for the first time to investigate the generation of peroxyapatite. Three oxygen generating compounds (peracetic acid, ammonium persulphate and hydrogen peroxide) were used in synthesis, but only ammonium persulphate and hydrogen peroxide produced peroxyapatite. The processing conditions need to optimize oxygen generation and crystallization to apatite to generate peroxyapatite. Hydrothermal processing is fast, cheap and simpler than the high temperature process for forming peroxyapatite.

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