

Comparison of nonstoichiometric and Mg-containing calcium-deficient hydroxyapatites obtained by enzymatic synthesis

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Experimental procedures

Enzymatic synthesis of calcium phosphate

Calcium glycerophosphate (30 mg) was dissolved in water (7 cm³) purified using an Aqualab S18 system (Russia) or in a Tris buffer (pH 8.5 at 25 °C, 7 cm³) containing 10 mM tris(hydroxymethyl)aminomethane hydrochloride (Tris-HCl), 100 mM NaCl, 10 mM MgCl₂ and 1 mM dithiothreitol (DTT) prepared by diluting a concentrated Tris buffer solution (SibEnzyme, Russia). Alkaline phosphatase from bovine kidney (Sigma) was added to a concentration of 2.6 g dm⁻³ (total enzymatic activity 5 U). The systems were incubated at 37 °C for 7 days. The precipitate was then separated by decantation, washed with water and dried at 60 °C to constant weight.

Determination of antimicrobial activity of calcium phosphate

Hydroxyapatite samples were tested for antimicrobial activity against the gram-negative bacteria *Escherichia coli* MG1651 and *Pseudomonas aeruginosa* 4.8.1, as well as the gram-positive bacteria *Bacillus subtilis* ATCC 6633 and *Bacillus cereus* ATCC 11778, using the agar well diffusion technique. A commercially available bacteriological reagent kit, ‘Dried Nutrient Medium for Determining the Sensitivity of Microorganisms to Antibacterial Drugs’ (Russia), was used, and the microorganism concentration corresponded to a turbidity standard of 0.5 according to McFarland.

Wells of equal diameter were then punched in the agar, and the test samples (100 mm³) were added at concentrations of 1, 0.5, 0.1 and 0.02 g dm⁻³. The growth inhibition zone was determined after 24 h of incubation at 37 °C. The sensitivity of the test microorganism to the test sample was determined by the diameter of the growth inhibition zone, expressed in millimeters (mm).

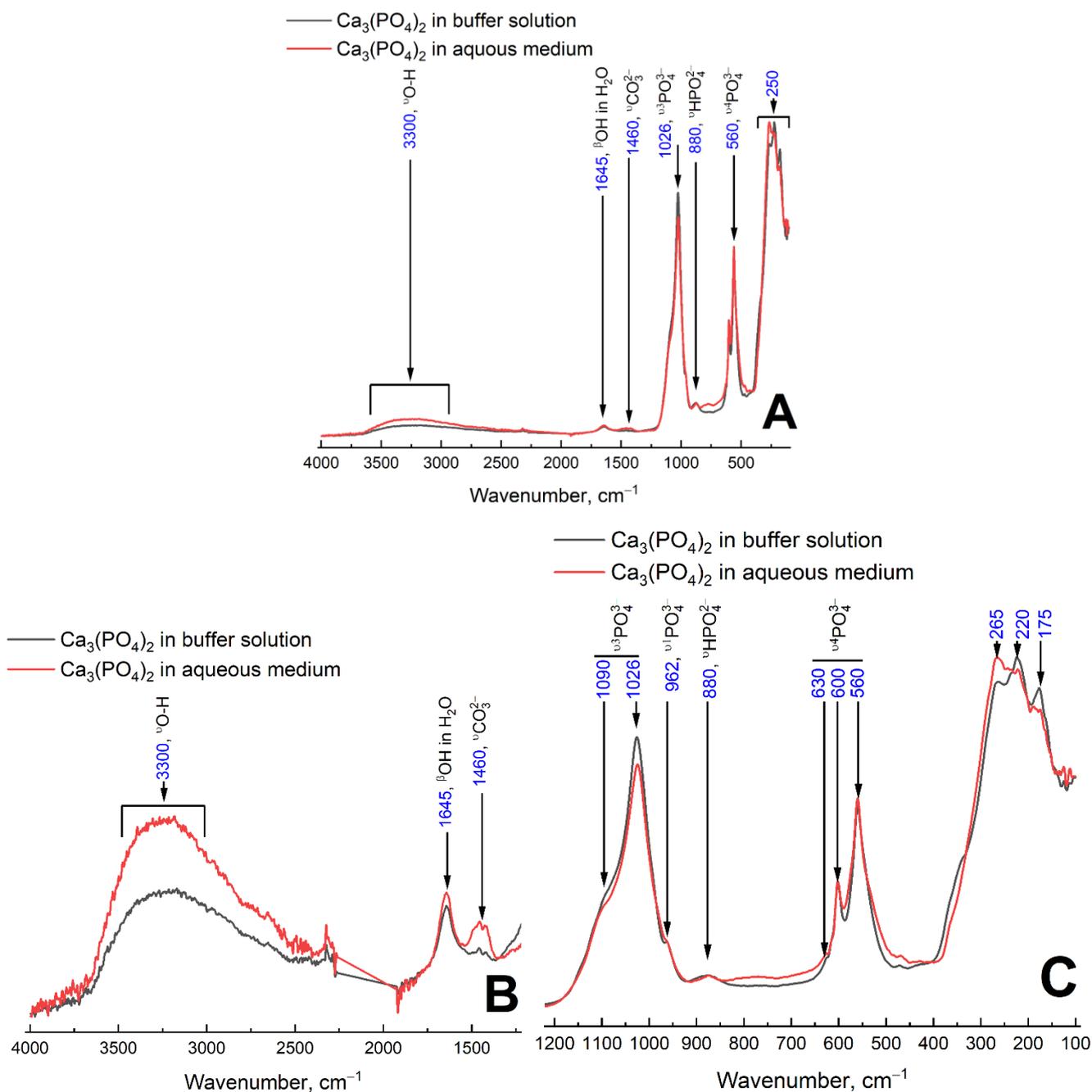


Figure S1 ATR-FTIR spectra of $\text{Ca}_3(\text{PO}_4)_2$ samples prepared in buffer solution and in aqueous medium, recorded in absorption mode in the ranges (A) from 4000 to 100 cm^{-1} , (B) from 4000 to 1200 cm^{-1} and (C) from 1200 to 100 cm^{-1} .