Copper and cerium co-substituted hydroxyapatite: powders synthesis and sintering

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Abstract. Copper- and cerium-substituted and copper-cerium co-substituted hydroxyapatite (HA) nanopowders were synthesized through aqueous precipitation method. The obtained materials were studied by XRD, SEM, BET absorption and dilatometry. The effects of the cation substitution for calcium ions in HA structure on the phase composition, lattice parameters, shrinkage and microstructure of the ceramics were studied and discussed. It was concluded that cerium ions incorporation in small amount (0.1 mol. %) contribute to a sintering process more than rather higher amount of copper ions (5 mol. %).

1. Introduction
Development of functionally-oriented biomaterials targeted for osteoplastical surgery devotes on bone reconstruction process and effective treatment. Eventually, the osteoconductive material for bone reconstruction has been the source of structural components while serves as matrix for new-bone formation. Various components could be involved into matrix material to design the specific function orientation. In proposed work we focused on development of the material for bone reconstruction that possessed antibacterial activity and luminescent properties. Bone defect treatment involves the extensive surgery that yields several complications such as postoperative infections, selection of systemic therapy and locale diagnostics. To find a way forward these problems we regard calcium hydroxyapatite (HA) ceramics with antibacterial and luminescent agents as functionally-oriented bone substitute. Being of great biocompatibility and bioactivity, HA ceramics provide high investigation value due to opportunities for improving mechanical and biological properties through the structure modifications. Cation and anion substitution in HA crystal lattice causes the micro-deformations and particle size decrease, rising of dissolution rate at the crystal-solution boundary. In the meantime, dopant type set the specific properties of biomaterial behavior in vivo [1-6].

Copper ions have been known as antimicrobial agent against a wide range of bacterial strains, particularly, *E. coli*, *St. aureus* [7-9]. Copper-substituted hydroxyapatites are also of interest in pigment synthesis due to copper ions availability in a range of different colors [10]. At the same time, cerium of number of rare earth elements have a significant ability to fluorescence and is used in fluorescent imaging [11-13]. Cerium ions possess bactericide effect against several gram-negative and gram-positive bacterial strains [14-17]. Cerium containing compounds are used in antibacterial and antitumor medicine and fluorescent visualization [18, 19]. HA structure allows the incorporation of wide range of ionic substitution from monovalent, divalent to trivalent and tetravalent ions [7, 15, 20-21]. Generally, the divalent copper ions stand into the Ca sites in HA structure and as a result the solid
solution of substitution forms [22]. Trivalent Ce ions have greater charge than Ca ions and close values of electronegativity and radius \((\text{Ce}^{3+}; \chi = 1.12, r = 1.03 \text{ Å} \text{ and for } \text{Cu}^{2+}; \chi = 1.0, r = 1.0 \text{ Å})\). Thus, the charge compensation can occur through cation vacancies and drifting of atoms in cation cites. On the other hand, copper ions have higher electronegativity and less radius than Ca (\((\text{Cu}^{2+}; \chi = 1.9, r = 0.8 \text{ Å})\) that could result in compensation in ionic sublattice of co-substituted HA structure.

Therefore, the bioactive functionally-oriented HA materials with complex of antibacterial and fluorescent properties is of great interest for developments of bone tissue engineering, cancer treatment and optical bioimaging diagnostic. Still, the Ca\textsuperscript{2+} co-substitution with Cu\textsuperscript{2+} and Ce\textsuperscript{3+} ions has not been studied before.

In this paper, we present the synthesis of copper- and cerium-substituted HA nanopowders in order to obtain antibacterial and luminescent properties. The effects of copper and cerium substitution for calcium ions in HA structure on the phase composition, lattice parameters, sintering process and microstructure of ceramics were studied and discussed.

2. Materials and methods

2.1. Materials and synthesis method

Pure HA, copper-substituted HA (Cu-HA), cerium-substituted HA (Ce-HA) and copper- and cerium co-substituted HA (Cu-Ce-HA) powders were prepared by precipitation technique from aqueous solutions of calcium nitrate tetrahydrate \((\text{Ca(NO}_3)_2\times4\text{H}_2\text{O})\), copper nitrate trihydrate \((\text{Cu(NO}_3)_2\times3\text{H}_2\text{O})\), cerium nitrate hexahydrate \((\text{Ce(NO}_3)_3\times6\text{H}_2\text{O})\) and ammonium hydroxide \((\text{NH}_4\text{HPO}_4)\). Concentration of all used solutions was 0.5 M. All reagents used in the work were corresponded to the qualification of “analytical grade” and higher. Designation of compositions and calculated content of dopants are presented in table (1). Pure HA was prepared by slowly addition of \((\text{NH}_4\text{HPO}_4)\) solution to \((\text{Ca(NO}_3)_2)\) solution while mixing. Cu-HA, Ce-HA and Cu-Ce-HA were prepared the same way, only \((\text{NH}_4\text{HPO}_4)\) solution was added to the mixed solution of calcium nitrate and dopant nitrate. During the synthesis pH level was kept at 11 ± 0.5 by addition of ammonia solution. Precipitate was filtered, washed by water and dried at 80 °C during 24 h. Then the compact cakes were crushed to the powder using mortar and pestle and sieved through a sieve with cell size of 100 µm.

| Sample | Cu\textsuperscript{2+}, mol. % | Ce\textsuperscript{3+}, mol. % | (Ca+Cu+Ce)/P |
|--------|-------------------------------|-------------------------------|----------------|
| HA     | 0                             | 0                             | 1.67           |
| Cu-HA  | 5                             | 0                             | 1.67           |
| Ce-HA  | 0                             | 0.1                           | 1.67           |
| Cu-Ce-HA | 5                       | 0.1                           | 1.67           |

2.2. Sample characterization

Phase composition, lattice parameters and average crystallite size of obtained materials were estimated by X-ray diffraction analysis (XRD, Shimadzu) using CuKα radiation (average wavelength \(\lambda =1.54183 \text{ Å}\)). Data were collected in 2θ range of 10–60 deg., step 20 of 0.02 deg. and counting time of 2 s/step. X-ray analyses were performed on the powered samples calcinated at 1300 °C. The lattice parameters \(a\) and \(c\) (Å) for hexagonal HA structure, were calculated from the diffraction peaks (002) and (211), respectively. The average crystallite size D (nm) of the powders was calculated from XRD data using the Scherrer’s equation. The specific surface area of the obtained powders was measured by BET method (TriStar 3000, Micromeritics). Sintering of materials was evaluated by measuring continuous shrinkage of pressed samples using horizontal dilatometer (DIL 402C Netzsch) with corundum equipment. Cylindrical samples (pressure 50 MPa) with a diameter of 5 mm and height of 4 mm were pressed for sintering study. Measuring was performed in argon atmosphere at constant heating rate of 10 °C/min to the temperature of 1400 °C with. Microstructure of the ceramics was
investigated by scanning electron microscope (SEM, Tescan Vega II SBU) with integrated Energy dispersive X-ray microanalyzer (EDX, INCA Energy).

3. Results
XRD analysis data of the synthesized and calcined powders indicated that all the materials consisted of the single crystalline phase of hydroxyapatite (Figure 1). Calculations of the lattice parameters revealed that the introduction of dopants into HA structure led to changes in crystal lattice (Table 2). Parameters \( a \) and \( c \) of Cu-HA \((a=9.416 \, \text{Å}, \, c=6.884 \, \text{Å})\) were less than for pure HA \((a=9.425 \, \text{Å}, \, c=6.887 \, \text{Å})\). This indicated the substitution of calcium with copper, since the ionic radius of copper (0.8 Å) was less than the ionic radius of calcium (1.06 Å). Parameters for Ce-HA \((a=9.427 \, \text{Å}, \, c=6.886 \, \text{Å})\) were insignificantly lowered than for pure HA, within error. Incorporation of cerium ions with larger radius (1.15 Å) in amount of 0.1 mol. % into the HA structure did not cause noticeable change of the HA crystal structure parameters. This could be explained by low amount of cerium and low method sensitivity, or by incomplete entry of the element into the structure. The co-substitution with copper and cerium ions of HA structure led to a decrease of parameter \( a \) and slightly increase of parameter \( c \) of the crystal lattice. This indicated an atoms redistribution in cationic/anionic HA sublattice.

![Figure 1. XRDs of obtained powders of substituted HA.](image)

| Table 2. Lattice parameters of substituted HA |
|---------------------------------------------|
| Sample          | \( a \) (Å) | \( c \) (Å) |
| HA              | 9,425       | 6,887       |
| Cu-HA           | 9,416       | 6,884       |
| Ce-HA           | 9,427       | 6,886       |
| Cu-Ce-HA        | 9,406       | 6,888       |
| \( \text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2 \) | 9,418       | 6,884       |

The plot of linear shrinkage vs the temperature for a compacted samples of the pure and substituted HA powders are presented on Figure 2, a. Its derivative curves are given in Figure 2, b. All examined powders had a similar surface area in range of 120-140 m\(^2\)/g, i.e. an average particle size was about 13-16 nm in approximation of sphere particle shape. Shrinkage measuring of the materials revealed that a slight intrinsic expansion of the powder compacts occurred only for pure HA and Cu-HA at 210-550 °C. The initial sintering state, when the interparticle neck formed and growth, started at 570 °C for both HA and Cu-HA powders and at 500 °C for both Ce-HA and Cu-Ce-HA powders. The active sintering process with a classic linear curve trend occurred as follows: at above 880-1000 °C for both HA and Cu-HA samples \((\Delta l/l_0=8,0\pm 22,6 \, \%)\), at 810-1010 °C for Ce-HA sample \((\Delta l/l_0=6,5\pm 22,6 \, \%)\) and at 770-955 °C for Cu-Ce-HA sample \((\Delta l/l_0=5,5\pm 19,5 \, \%)\). During this intermediate stage the sintering rate is maximum. At the end of this stage for each sample the shrinkage curve had been gentler in slope corresponded to slowing down of the sintering stage. At 1300 °C, the traditional temperature of the end of HA sintering, shrinkage value was measured as follows: 26,6 % for HA, 24,7 % for Cu-HA and 25,1 % for both Ce-HA and Cu-Ce-HA. Based on dilatometry data it could be
noted that copper doping resulted in light decrease of HA sintering rate due to reducing of diffusion processes [23]. Cerium ions significantly decreased the sintering rate (Figure 3, b). While sintering is the mass transfer process, the sintering rate and other parameters depended on material’s mass. So, if molar mass of Ce (M=140,12 g/mol) is much higher than for Ca (M=40,08 g/mol), its atom diffusion is restrained in compare with Ca-atoms. Co-substituted Cu-Ce-HA demonstrate the same sintering behavior as Ce-HA and low sintering rate. Also some widening took place in the plot of Cu-Ce-HA shrinkage rate vs the temperature pointed to an additional process of phase allocation or lattice deformation during thermal activation. So it could be conclude that cerium ions incorporation prevented mostly the sintering kinetic process of HA-ceramics.

![Figure 2](image-url)

**Figure 2.** Linear shrinkage of obtained HAs vs the temperature (a) and shrinkage rate (b).

Figure 3 demonstrates the structure of spalling surface of ceramic substituted HAs samples. Pure HA ceramics consisted of crystals from 2 to 6 μm in size with strong boundaries (Figure 3, a). There was small amount of intercrystalline pores of average size of 0.5-1.0 μm. Copper incorporation into HA resulted in presence of irregularity of crystal shape and more friable surface. Also pores became bigger in size, number of pores has increased. The introduction of cerium into HA structure didn’t significantly change the microstructure of HA ceramic. Cu-Ce-HA ceramics characterized by roughness of the crystal surface. Crystals had irregular shape and size from 2 to 5 μm. Intercrystalline pores are large and numerous. Thus, the copper substitution of HA ceramics led to irregular crystal structure with rising of number of intercrystalline pores. Cerium introduction into HA structure didn’t significantly affect the crystal structure, but inhibited sintering process and reduced the shrinkage of the materials.
4. Conclusion
In the work there were synthesized powders of Cu-HA, Ce-HA and Ce-Cu-HA with calculated copper content of 5 mol. %, cerium content of 0.1 mol. %, and Cu/Ce of 5/0.1 mol. %. The synthesis was performed through aqueous precipitation method. The obtained powders of cation substituted HAs were of average size of 13-16 nm. Materials were studied by XRD, SEM, BET absorption and dilatometry. It was found that the introduction of copper into HA structure reduced lattice parameters $a$ and $c$ due to smaller ion radius Cu than Ca. Also it led to irregular crystal structure with increase of number of intercrystalline pores in the HA ceramics. Incorporation of Ce ions with larger radius (1,15 Å) in amount of 0,1 mol. % into the HA structure did not change the crystal structure parameters. Ce didn't significantly affect the ceramics microstructure, but rather inhibited sintering process and reduced the shrinkage of the material. The co-substitution of HA with Cu and Ce ions led to a decrease of parameter $a$ and slightly increase of parameter $c$, that caused for atoms redistribution in cationic/anionic HA sublattice. The sintering behavior of Cu-Ce-HA was similar to Ce-HA. Thus, small Ce amount (0,1 mol. %) contribute to a sintering process more than rather higher amount of copper ions (5 mol. %).

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