



RESEARCH ON HYDROXYAPATITE BASED COMPOSITE MATERIALS

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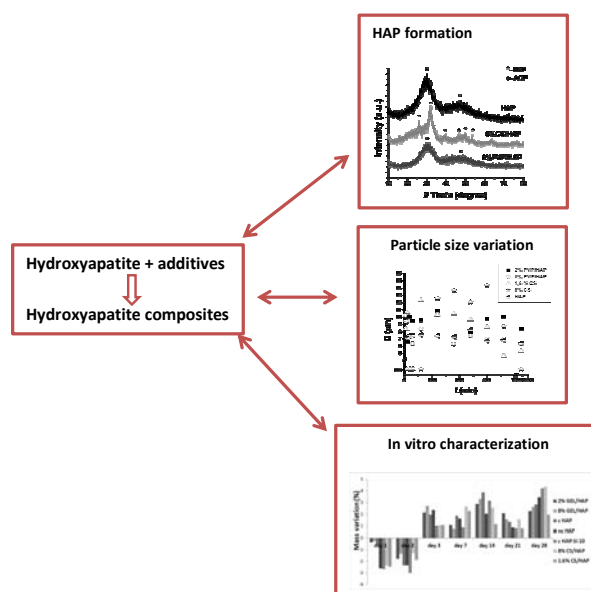
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The application of hydroxyapatite (HAP) as a substrate to stimulate bone ingrowth remains limited due to its extreme brittleness. Many biodegradable polymers (chitosan, gelatin, poly (vinyl-pyrrolidone)) with more flexible mechanical properties have been combined to create polymer/HAP composites. These materials influence the nucleation process and particle growth during HAP synthesis. This paper presents the influence of different additives on particle size variation during composite synthesis. To predict a potential bone-bonding behavior through new apatite layer formation the immersion in simulated body fluid (SBF) is a reliable testing method. SBF soaking results revealed that all the prepared hydroxyapatite composites are biologically active.



INTRODUCTION

Hydroxyapatite ($\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$ -HAP) is the main inorganic component of bones and teeth. HAP has been extensively used as an implant material for bone substitute owing to its excellent osteoconductive properties.¹⁻³ The numbers of medical applications of HAP are limited, primarily due to its relatively poor mechanical properties.⁴

Small amounts of biopolymers and/or silicon introduced during HAP synthesis improve the mechanical properties, increase the composites biocompatibility,^{5,6} and the particle size could be also influenced.^{7,8} Particle size determines the materials physical and chemical properties, being a valuable indicator of quality and performance. Nano sized hydroxyapatite has increased properties as: higher specific surface area,⁹ improved strength,

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hardness, ductility, tensile properties¹⁰ and thermal stability, resistance to tear and higher reactivity.^{11, 12} Biopolymer like chitosan (CS),¹⁰ gelatin (GEL)¹³ and poly (vinyl-pyrrolidone) (PVP)¹⁴ are used as additives to reproduce the properties of the natural bone, that is due to the unique hierarchical integration of HAP within the collagen matrix.^{13, 15}

Testing in simulated body fluid (SBF) is an excellent approach¹⁴ for *in vitro* biocompatibility study, which is important in predicting a potential bone-bonding behavior through the apatite layer formation on its surface.¹⁶ The role of SBF testing is to simulate and study the inorganic subsystem of biomaterial and human body plasma interaction.

The *main goal* of this paper is to present a global method for hydroxyapatite composites preparation, to underline the effect of biopolymer addition on particle size variation and the *in vitro* characterization.

RESULTS AND DISCUSSION

1. Formation of HAP

The evolution of crystalline phases with reaction time was studied by X-ray diffraction measurements. The total synthesis time was 22 hours and the crystallinity of HAP during preparation was examined. For all the studied materials calcium phosphate (CaP) or amorphous calcium phosphate (ACP) phase formation appears in the initial stages of the synthesis and then their transformation into a more stable HAP phase occurs.¹⁷ The diffractogram of pure hydroxyapatite shows that after 6 hours (Fig. 1B) a crystalline HAP structure is formed.

In the case of CS/HAP, at the beginning of the reaction, a poorly crystalline hydroxyapatite phase appears, that after 6 hours transforms into ACP, than recrystallizes into HAP. The poorly crystalline phase formation in the early stage of reaction can be explained by changes in the reactant's solubility due to the presence of chitosan.⁷ After 22 hours (Figure 1C) a single-phase apatite formation was observed and no secondary phase was detected. At PVP/HAP formation there are no major differences between the spectra's of 30 minutes and 6 hours, leading to a crystalline composite after 22 hours.⁷

2. Particle size variation

The particle size distribution was measured with Shimadzu SALD-7101 micro- and nano particle analyzer. In Fig. 2 the particle size variation is represented during synthesis for hydroxyapatite and HAP based composites. In the case of HAP, in the first six hours the average particle size gradually increases reaching the micrometer domain and after then decreases. This phenomenon can be explained by the agglomeration and segregation of HAP particles during preparation.⁷ Macromolecules as additive materials act as a soft temporary template or nucleation centers to modulate the morphology and increase the crystallinity of HAP.^{18, 19} That is why the surface-regulating PVP is used as a capping agent to regulate the nucleation and crystal growth of HAP crystals. The effects of polymer amount on the physical properties of HAP crystals were studied. The size and shape development of HAP nanocrystals precipitated in an aqueous solution of PVP is inversely related to the polymer amount (*i.e.* the smallest particle size was observed with the highest PVP amount), similar to the results published by Zhou *et al.*²⁰ In the PVP structure,^{21, 22} the O—H groups are located in abundance on the surface of HAP crystals, this why hydrogen bonds can be formed between PVP and HAP, which prevents nanoparticle aggregation. So, the particle size in case of low polymer concentration is higher than in the case of pure HAP (see Figure 2A), because the polymer promotes particle growth. At higher PVP concentration a larger number of reaction sites are assured leading to a higher number of HAP nuclei, and therefore a smaller particle size²⁰ – a growth-blocking action occurs. In the case of CS/HAP if the CS amount is increased, a decrease of particle size occurs. The fluctuation in the average particle size variation is attributed to the alternation of two processes: nucleation and crystal growth. After the particles reach a critical mass, they disintegrate and in order to reduce the surface tension they stick again together, until a balance of the processes is achieved.^{19, 23}

In the case of HAP-Si 10% the particle agglomeration process does not occur, the average particle size is constantly situated at the value of 20 nm (Fig. 2B). Silica addition inhibits crystal growth and also reduces the agglomeration tendency.²⁴

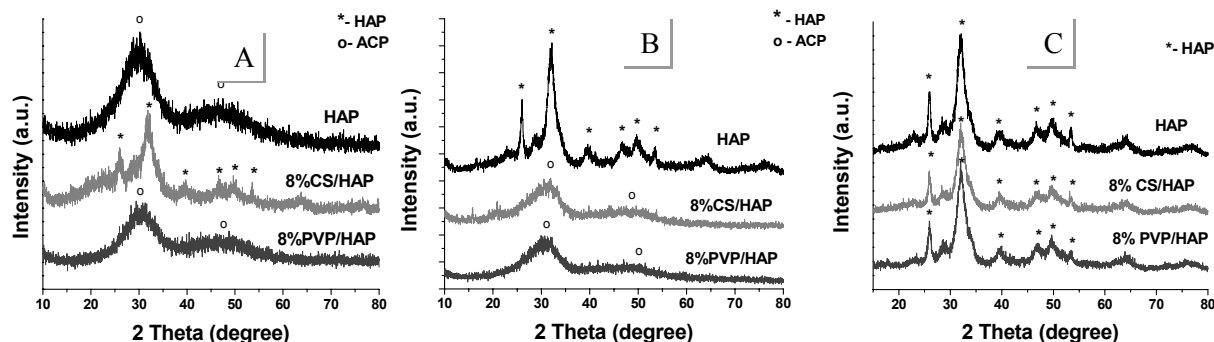


Fig. 1 – XRD spectra of HAP and their composites: A- after 30 min, B- after 6 h, C- after 22 h.

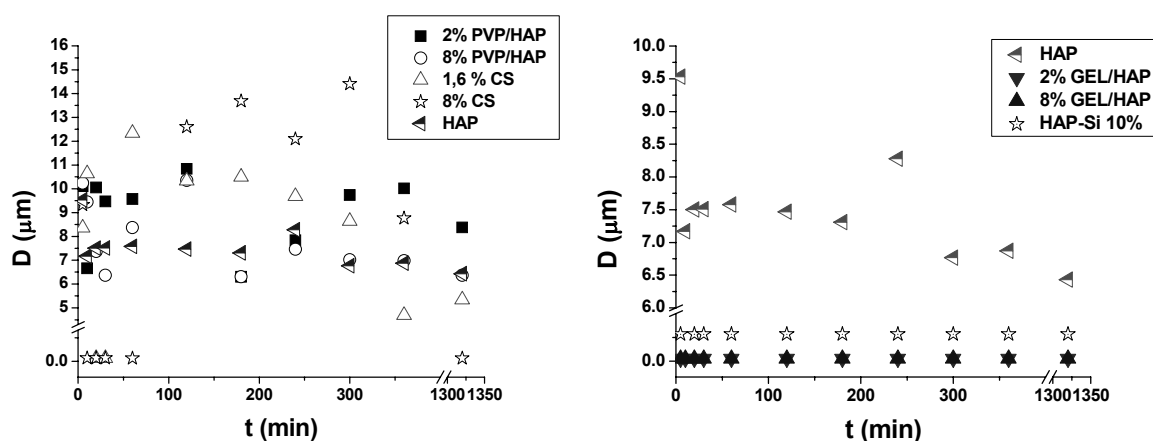


Fig. 2 – Average particle size variation for HAP and HAP based composites during synthesis time.

Gelatin is an amphoteric polyelectrolyte because the gelatin chain contains both anionic and cationic groups, which can adsorb ions. The adsorption could be driven by electrostatic or/and by hydrophobic interaction, depending on the nature of surface and the medium. In the gelatin aqueous solution dissociation of ionic bonds occurs because most of carboxyl groups are changed into a protonated form, resulting an interaction between Ca^{2+} ions in the solution and $\text{R}-(\text{COO})^-$ ions of the gelatin molecules. Increasing the initial gelatin concentration the gelatin macromolecules interaction sites are also increasing.^{18,25,26} The interactions among calcium complexes and the growing of HAP nanocrystals are predominantly determined by the length scales of GEL and the critical reaction distance between GEL and Ca^{2+} ions. The critical reaction distance is controlled by the concentration of GEL macromolecules. This organization process manipulates microstructure and physical properties of HAP–GEL nanocomposites.²⁷

3. In vitro bioactivity testing

When a material is incubated in SBF solution, a new apatite layer appears on the surface of the pellets through a sequence of chemical reactions

like spontaneous precipitation, nucleation and growth of calcium phosphate. It has been suggested that surface chemistry plays an important role in this process and even the functional groups of materials have a large effect on the bone-bonding property.²⁸

The formation of a new apatite layer was studied by the immersion of composites in simulated body fluid. In the first 2 days of immersion the mass of HAP based material pastilles decreased, caused by the dissolution of HAP^{29} into Ca^{2+} , PO_4^{3-} and OH^- ions (Fig. 3).

The graphic reveals that in the first 1-2 days all the materials suffer a mass decay (2-3 mass%) with further increase/decrease in function of the soaking time, suggesting a continuous precipitation of the bone-like apatite. CS was reported to have a high bioactivity due to the fast formation of an $\text{Ca}_5(\text{PO}_4)_3\text{OH}$ layer on its surface after soaking in simulated body fluid solutions. The HAP-Si activity is increased because it is known that the silanol groups on the silicate surface can provide favorable sites for apatite nucleation.³⁰ The weight loss is more reduced for the chitosan and silica-hydroxyapatite composites, so the introduction of chitosan and silicon increases the *in vitro* activity of the hydroxyapatite composites.

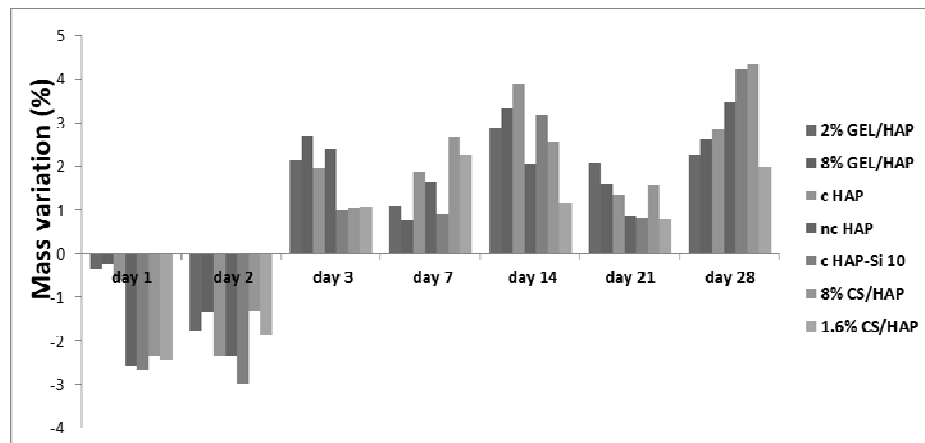


Fig. 3 – The mass variation of the hydroxyapatite composites during 28 days of SBF soaking.

EXPERIMENTAL

1. Materials preparation

The HAP and its composites were prepared by precipitation method.³¹ After synthesis HAP and HAP-Si 10 were calcined at 1000°C (16°C/min heating rate).

The following materials were prepared: non calcined HAP (ncHAP), calcined HAP (cHAP), calcined HAP containing 10

wt% SiO₂ (cHAP-Si 10), non-calcined HAP containing 2 wt% gelatin (2% GEL/HAP), non-calcined HAP containing 8 wt% gelatin (8% GEL/HAP), non-calcined HAP containing 1.6 wt% chitosan (1.6% CS/HAP), non-calcined HAP containing 8 wt% chitosan (8% CS/HAP), non-calcined HAP containing 2 wt% PVP (2% PVP/HAP) and non-calcined HAP containing 8 wt% PVP (8%PVP/HAP).

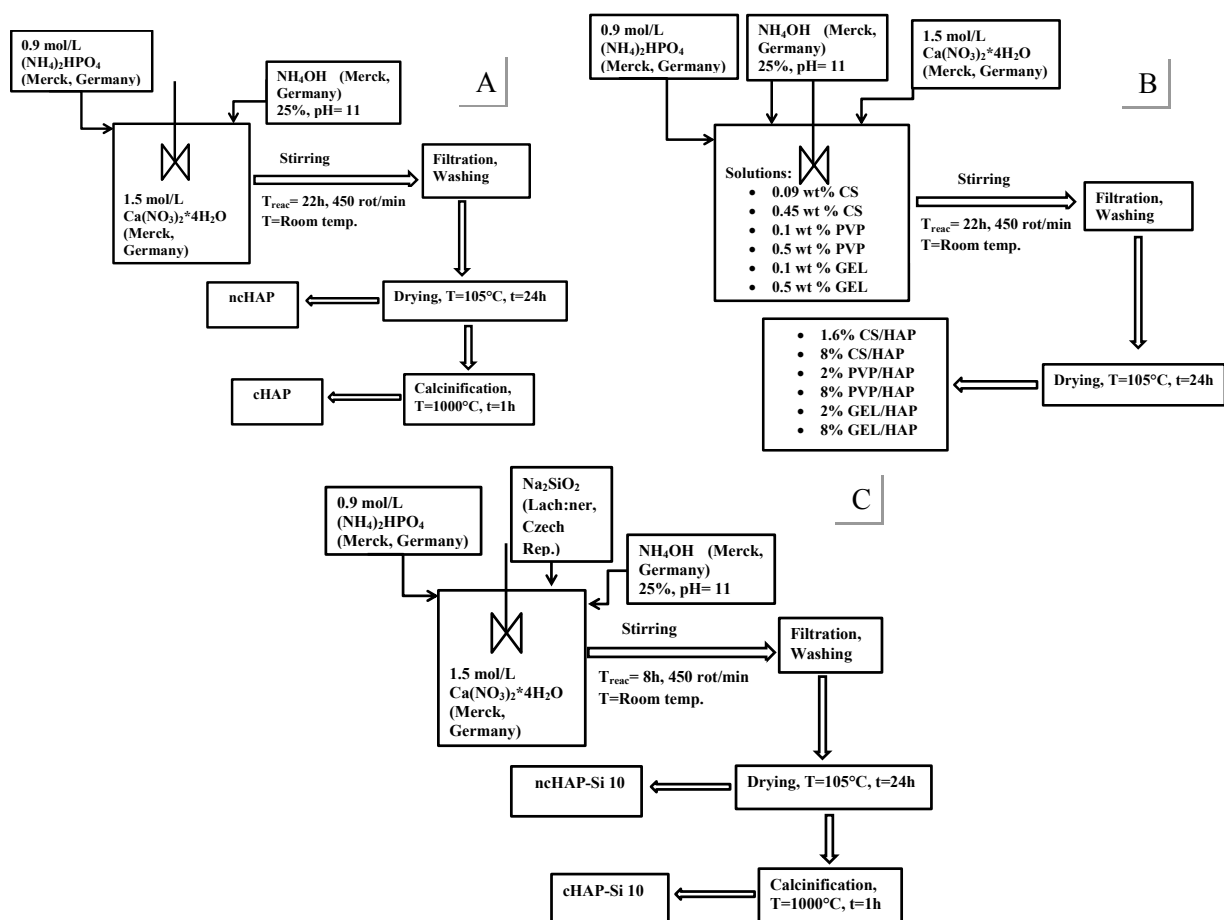


Fig. 4 – Preparation schemes of HAP and HAP based composites: A- HAP, B- CS/HAP, PVP/HAP, GEL/HAP, C- HAP-Si 10.

In Fig. 4 the preparation schemes of the HAP materials are shown. 0.09 wt% and 0.45 wt% CS, respectively 0.1 and 0.5 wt% GEL and PVP stock solutions were prepared by dissolving appropriate quantities of chitosan (medium viscosity, MW = 3800 Da, Merck, Germany), gelatin and PVP in double distilled water. The final CS concentration in the composite is 1.6, respectively 8 wt%. The gelatin and PVP composites contain 2% and 8 wt% biopolymer. Silica doped hydroxyapatite contains 10 wt% SiO₂.

2. In vitro biological activity measurements in simulated body fluid

The SBF solution was prepared as described Juhasz *et al.*³², reagent grade NaCl (Reactivul, Roumania), NaHCO₃ (Merck, Germany), KCl (Reactivul, Roumania), K₂HPO₄·3H₂O (Lach:ner, Czech Republic), MgCl₂·6H₂O (Lach:ner, Czech Republic), CaCl₂ (Nordic, Roumania) and Na₂SO₄ (Reactivul, Roumania) in distilled water.¹³ The solution was buffered to obtain pH between 7.25-7.40 with hydrochloric acid (Reactivul, Roumania) and TRIS [(CH₂OH)₃CNH₂] (Merck, Germany) at 37 °C. The pH was monitored with an Electrode SenTix 41-3 pH electrode. The used simulated body fluid exhibits inorganic ion concentrations nearly equal to those of human blood plasma.^{24, 33} The hydroxyapatite-based materials (40 mg) were introduced into 20 mL SBF for 28 days and weight variation was studied.

CONCLUSIONS

Hydroxyapatite based composites have been prepared with different additive materials and these composites show different properties compared to pure hydroxyapatite. The presence of additive materials in the composites influences the: formation of apatite's structure, average particle size and *in vitro* activity.

In order to achieve the highest efficiency for a specific application, the strong relation between synthesis parameters (silica doping, bio-polymer addition) and materials characteristics is essential to be taken into consideration. Considering that previous allegation, it is possible to control the properties of HAP based materials already by their preparation. Based on the results presented in this paper the *in vitro* properties of HAP can be controlled by biopolymer addition, and this way materials with more suitable characteristics for biomedical applications can be obtained.

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