Effect of ions concentration in buffer solutions on the nucleation of hydroxyapatite surface on the octacalcium phosphate granules

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Abstract. Octacalcium phosphate granules were subsequently immersed for different lengths of time from 1 day to 44 days at constant temperature 37±0.4°C in solutions, simulating the extracellular body fluids: Supersaturated Calcification Solution (SCS), Simulated Body Fluid (SBF), based on the ISO-10993 "Biological Evaluation of Medical Devices" standard. The role of buffer’s systems pH-value, time of the material exposure and calcium and phosphorus ionic concentrations were analysed with the main focus on kinetics of degradation, changes in the morphological characteristics and phase composition of octacalcium phosphate samples.

1. Introduction
The process of biological bones mineralization takes place in the intercellular body fluids. Hydroxyapatite (HA) is a major mineral component of bone tissue, but the mineralization starts from it’s precursor – octacalcium phosphate (OCP) that is why it is of special interest in bone regeneration due to it’s biocompatibility and biodegradability. This study was performed to examine in vitro stability of OCP granules. In the present investigation, the role of immersion OCP granules in static physiological solutions with a constant temperature of 37±1 °C during 44 days was examined with the focus on the composition and morphologies changes after every chosen period. Octacalcium phosphate (Ca$_8$H$_2$(PO$_4$)$_6$·5H$_2$O) has the osteogenic capability and rate of resorption, close to speed of new bone tissue formation. However, the main direction in tissue development is creation bio-engineering structures with drugs or growth factors addition for directed bone healing, disease treatment and improvement implant’s osteoinduction and osteoconduction. Biomimetic deposition method suits the physiological temperature and pH requirements for infiltration biological agents [1]. Although this method was frequently used with hydroxyapatite (HA) [2,3] and many other materials [4], there is no previous report of using it on OCP granules.

The synthesis of pure OCP must be carried out under strict controlled conditions to prevent hydrolyzation OCP to HA [5]. Temperature, pH, solid-to-liquid ratio, Ca/P ratio, ion composition and concentration effect on OCP [6].

2. Materials and methods
In this work, octacalcium phosphate was prepared by chemical transformation method in the sodium acetate solution from α-TCP granules, which were synthesized by precipitation from solutions of calcium acetate (0.04 mol/L) and sodium hydrogen phosphate (0.04 mol/L). The pH value and
solution temperature varied in the physiological ranges of 5.0–6.5 and 37–90°C, respectively [7,8]. For this study, we used the samples from 1000 to 2000 μm. Buffer solutions, which are SCS and SBF, were prepared by dissolving appropriate quantities of the chemicals in deionized water. Reagents were added one by one after completely dissolved previous reagent in 800 ml of water [9,10]. The chemical composition, pH values and ions concentration of the buffer solutions were determined before the experiment and detailed in Table 1. The choice of solutions was based on physiological pH-value (≈ 7.4), temperature (37 °C), buffers system composition and ions concentration. According to previous research results, buffer solutions should have pH about 7.0 – 7.6 and Ca\(^{2+}\) (≈ 1.35 mM) concentration have to be lower than (PO\(_4\))\(^{3–}\) (≈ 2.0 mM) for launch of the OCP transformation to HA [11].

The experiment was carried out in closed systems: each sample was exposed to the solutions under static conditions at 37 °C ± 0.4 °C for determinate period of time: from 1 day up to 44 days. The samples one by one were carefully immersed in 50 ml volume of each solution for 1 g of solid material. After exposure, the samples were carefully washed off by distilled water and dried at the same temperature. The Ca\(^{2+}\) and (PO\(_4\))\(^{3–}\) concentrations were determined by atomic emission spectroscopy (AES), solutions pH-value was measured using pH meter. Mass measurements of OCP granules were carried out before and after soaking in closed containers for comparison solubility rate and degradation behavior depending on time. Assessment of the degradation kinetics was carried out in the simulated extracellular body fluids according to Biological evaluation of medical devices - Part 14: Identification and quantification of degradation products from ceramics [12]. Complementary information about the surface morphology transformations during samples immersion in buffer systems was examined by scanning electron microscopy (SEM) Tescan VEGA II (Czech), previously covered with gold by Q150R Quorum Technologies (England). X-ray powder diffraction (XRD) studies were performed with a SHIMADZU XRD-6000 diffractometer (Japan) with CuKα radiation (λ = 1.5418 Å), 40 kV and 200 mA. The angular interval 2Θ analyzed was from 10 to 60, with 2º/min for the qualitative analysis. The phases were identified resorting to JSPDS. The Fourier transform Infrared spectroscopy (FT-IR) were recorded by Nikolet Avatar 330 spectrophotometer (USA) in the 4000–400 cm\(^{-1}\) wavelength region. The KBr pellet technique was used with 1 mg of powder in 50 mg of spectroscopic-grade KBr. Chemical analysis was performed by inductively coupled plasma atomic emission spectroscopy (AES) in an ULTIMA 2 instrument (France). Weighed portions of the studied materials were dissolved in 10 % hydrochloric acid. The main interest is concentrations changes of calcium and phosphate ions over time of immersion.

### 3. Results and discussion

Figure 1 shows the X-ray spectra of the OCP used, showing only OCP and HA phase diffraction lines. The presence of HA as an impurity in OCP associated with the method of obtaining and properties of the material.
The powder X-ray diffraction patterns of the samples from SCS solutions for each immersion duration are reported in Figure 2. The phase ratio shifts towards growing HA phase compared to OCP with increasing exposure time. This transformation is possible only in supersaturated solution, when Ca$^{2+}$ and (PO$_4$)$_3^{-}$ ions concentration is sufficient for sedimentation of HA on the surface layer. Ions concentration increases possible due to high solubility of OCP [13].

In the SCS solution, granule composition ratio changes are noticeable in favour of an increase in the hydroxyapatite phase, which indicates the occurrence of recrystallization process at the phase boundaries. In the SBF buffer system, which is close to ionic composition of blood plasma material modification processes are similar with SCS solution. IR-spectroscopy results presented in Figure 3.
revealed specific infrared spectra of octacalcium phosphate. The spectrum of sample exhibits a strong bands of the $\nu_1$ mode of $(\text{HPO}_4)^{2-}$ groups at 1020 cm$^{-1}$ and the $\nu_4$ mode of the $(\text{PO}_4)^{3-}$ groups at 601 cm$^{-1}$. A typical feature of OCP observed in the spectrum of this sample is the presence of intense bands at 1138–1023 cm$^{-1}$ corresponding to the $\nu_3$ mode of $(\text{HPO}_4)^{2-}$ and $(\text{PO}_4)^{3-}$ and medium intensity bands at 601–561 cm$^{-1}$, which is the $\nu_4$ mode of $(\text{PO}_4)^{3-}$. The band at 856 cm$^{-1}$ was shared by $(\text{HPO}_4)^{2-}$ and $(\text{CO}_3)^{2-}$ due to presence CO$_2$ from the atmosphere.

IR-spectra of the samples after immersion in SCS Fig. 3 (a) and SBF Fig. 3 (b) liquids. The comparison of bands intensity changing depending on immersion duration illustrate that $(\text{PO}_4)^{3-}$ groups become more polar as the absorption band intensity is greater in different soaking period, which indicates the phase changes in the material.

The crystal morphology of OCP granules and it’s appropriate size for the experiment requirements presented in Figure 4. Crystals of OCP samples is composed of needles, which are less than 1 μm thick and up to 10 μm long. The phase changes in OCP granules surface layer on Figure 4. in SCS (b) and SBF (c) solutions are well seen. The structure of crystals is heterogeneous. Most of the surface is similar to HA, but some parts are still looks like OCP. According to chemical analysis performed on
Figure 5, it is seen that maximum changes occur in the first two days of the experiment. A necessary sufficient concentration of the ions for recrystallization process is initially found in SCS. Ca and (PO$_4$)$_3^-$ concentration in it decreased by more than 20% of the original concentration after soaking for 2 days, because the recrystallization of OCP started immediately. By the 3rd day of the experiment ions exchange with the rest material is stabilized and tends to equilibrium. In contrast, in a bipolar SBF solution there are not enough phosphorus ions. Therefore, the ion exchange between the surface layer of OCP granules and the solution leads to simultaneously degradation and recrystallization processes, as the calcium concentration rapidly decreases, while phosphorus ions gradually increase.

![Figure 5](image.png)

**Figure 5.** Ca$^{2+}$ and (PO$_4$)$_3^-$ concentrations in the (a) SCS and (b) SBF solutions as a function of soaking time.

4. Conclusions

The influence of immersion time in buffer solutions of various ionic composition, simulating the extracellular body fluids, on the kinetics of degradation, phase composition and morphological characteristics of calcium phosphate phases, including the microstructure and properties of material are reported. Interconnection between fluids ion concentration value and intensity XRD peaks of samples before and after immersion showed that recrystallization process on the surface layer is going faster in supersaturated solutions with sufficient concentration of Ca$^{2+}$ and (PO$_4$)$_3^-$ ions. Summing up all the research results, Supersaturated Calcification Solution buffer system has optimal characteristics for infiltration biological agents and drugs. The X-ray data evidenced the formation of the HA peaks on the OCP species with the increase of the immersion time. Scanning electron microscopy reveals the formation of heterogeneous and uneven distribution of spherical hydroxyapatite particles in SCS and SBF solutions by 44th day. The calcium and phosphor ionic concentration dependence from the OCP material exposure duration indicated that the reprecipitation process most quickly occurs in SCS. The results show that applicants can be infiltrated in material using buffer systems. Based on all the results presented above, it can be concluded that the SCS solution is optimal for the biomimetic deposition method for infiltration of various biogenic factors and drugs.

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