



*Short Communication*

## Determination of the Ca/P ratio in calcium phosphates during the precipitation of hydroxyapatite using X-ray diffractometry

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### Abstract

*The applicability of the X-ray powder diffraction method to the determination of phase composition and Ca/P ratio in precipitates during the nitrous wet synthesis of hydroxyapatite (HA) has been shown. The plotted dependences of the phase composition and the Ca/P ratio on the synthesis time can be used as initial data for the development of new and simple processing routes of calcium phosphate ceramics based on HA of any desired composition.*

**Keywords:** *bioceramics, apatite, wet synthesis, characterization, X-ray diffraction*

### I. Introduction

Ceramics of hydroxyapatite, HA,  $\beta$ -tricalcium phosphate,  $\beta$ -TCP, and their mixtures are successfully used for medical purposes [1,2]. The initial material for preparation of the ceramics is a HA-powder. The wet synthesis is declared as the most simple, cheap and suitable for industry of the methods developed [3]. The first precipitate emerged at the wet synthesis is an amorphous calcium phosphate, ACP. The ACP transforms to HA as the synthesis proceeds. A number of processing variables affects the conversion, and this interrelation has been comprehensively studied. The variables highly affect the physico-chemical characteristics of the HA powder, and the functional properties of HA-based ceramics depend on these characteristics [3]. Beside necessity in ceramics fabrication, results of such studies are of paramount importance for health science because the ACP precipitation and its conversion to HA mimics the formation of biogenic apatite and its alteration during life of a human being [1,2].

The mechanism of ACP to HA conversion has not been revealed until now. According to an earlier but still considering conclusion, the conversion is a solution-mediated process [4–7]. However, in later and recent studies, especially those performed using modern techniques,

different approaches have been proposed [8–11], in particular, there are sound results on the HA crystallization as an internal rearrangement or diffusion process in the particles constituting the ACP phase [12–14].

Essential data in such studies is derived from Ca/P ratio in the precipitates during the whole crystallization process. The ratio is usually determined by solution chemical analyses, atomic absorption spectroscopy or electron-microprobe spectroscopy [15]. All of the methods have drawbacks. The first two methods are time-consuming, and the Ca and P concentrations cannot be determined simultaneously. Besides, a substantial experimental error can appear during the phosphate concentration measurement by the colorimetry because the molybdenum complex varies with temperature and time. The third method is designated for measurements on a microscopic scale, and the obtained values are not accurate. A fast and reliable technique of determining the Ca/P ratio based on X-ray diffraction analysis, XRD, was proposed [16–18]. It was successfully applied for the measurements in powders of calcium-deficient hydroxyapatites, CdHAs [17]. It was further shown that for  $1.5 < \text{Ca/P} < 1.67$  the XRD method is even more accurate than the classical wet methods [18]. As during the conversion in the conventional (nitrous) wet synthesis, the Ca/P ratio usually changes from 1.5 (ACP,  $\text{Ca}_3(\text{PO}_4)_2 \cdot x\text{H}_2\text{O}$ ) to 1.67 (HA) [3,5], the XRD method is firstly examined in the monitoring of the Ca/P ratio in precipitates during ACP to HA conversion.

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## II. Experimental

The nitrous synthesis [3,4] for preparation of calcium phosphate powders was performed. Briefly, a calcium nitrate solution was rapidly added (900 ml/min) into an ammonia hydrophosphate solution under continuous stirring at Ca/P = 1.67, pH = 11–12 (adjusted and maintained by adding a  $\text{NH}_4\text{OH}$  solution), and 21 °C in the mixture; other variables are detailed elsewhere [19].

Aliquots were immediately separated after a precipitate was formed (about 2 min after the mixing) and then every 2 h during 32 h; the last of the reaction slurry was withdrawn 33 h after the beginning of synthesis. The aliquots were divided into two parts. The first part in as-separated state was dried in an oven at 60–70 °C for 24 h. The second part was washed with abundance of distilled water and centrifuged three times, and then dried at 60–70 °C for 72 h. All dried products were ground into fine powders and subjected to analyses before and after firing in a muffle oven at 1000 °C for 1 h.

The samples were characterized by X-ray powder diffraction (XRD; DRON-2 diffractometer, USSR,  $\text{CuK}_\alpha$  radiation). The phases were identified using the International Centre for Diffraction Data powder diffraction file (№ 9-432 for HA and № 9-169 for  $\beta$ -TCP). First, the HA /  $\beta$ -TCP ratio was found in a calcined biphasic calcium phosphate using a calibration curve for different HA /  $\beta$ -TCP mixtures [16]. After the determination of the phase proportion, the Ca/P atomic ratio of the initial nonstoichiometric powder, subjected to the calcination, was calculated (in the range of  $1.5 < \text{Ca/P} < 1.67$ ) from the weight percent of  $\beta$ -TCP,  $x$ , as

$$\text{Ca/P} = \frac{3x + 10(100 - x) M_{\beta\text{-TCP}} / M_{\text{HA}}}{2x + 6(100 - x) M_{\beta\text{-TCP}} / M_{\text{HA}}}$$

where  $M_{\text{HA}}$  and  $M_{\beta\text{-TCP}}$  are the molar weights of HA and  $\beta$ -TCP (1004.64 and 310.18 g/mol, respectively).

## III. Results and discussion

The samples separated from the precipitate in 2 min, 2, 4 and 6 hours after the mixing and fired at 1000 °C solely consisted of HA if the samples were not subjected to the traditional centrifugation-washing-drying procedure [19]. This was expected because the molar Ca/P ratio in the reaction mixture was desired as that of HA (1.67). However, if traditional centrifugation-washing-drying procedure was performed, some free  $\text{Ca}^{2+}$  was removed together with an aliquot, and the sample after firing yielded either a  $\beta$ -TCP phase (in precipitates of a few minutes lifetime), or a biphasic mixture with increased HA /  $\beta$ -TCP ratio as the precipitate lifetime increased (right up to the end of crystallization). This interrelation was quantitatively studied for the whole crystallization process. As a result,  $\beta$ -TCP was the major phase in the biphasic precipitate until 2 h lifetime, mixtures of  $\beta$ -TCP and HA with increasing HA /  $\beta$ -TCP ratio were found in

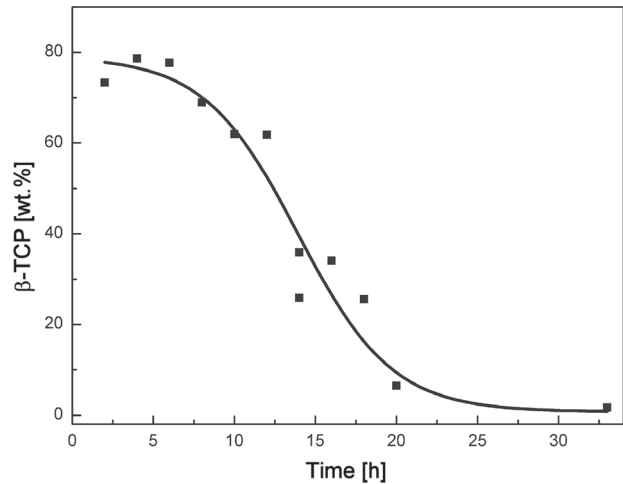


Figure 1. Dependence of the composition in a precipitate on the synthesis time

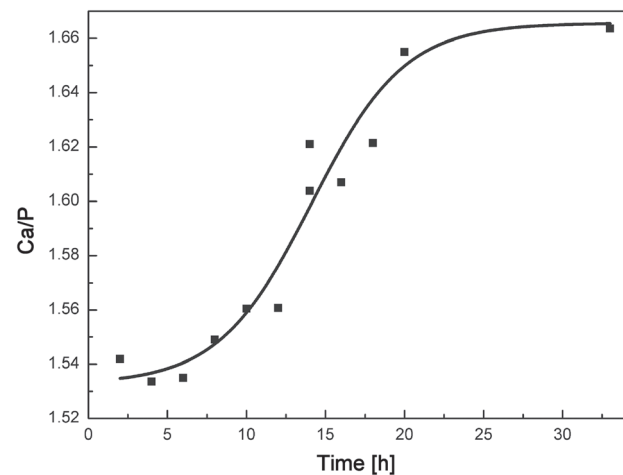


Figure 2. Dependence of the Ca/P ratio in a precipitate on the synthesis time

the precipitates of 2–32 h lifetimes, and pure HA was only detected in the precipitates of 32 h and longer lifetimes (Fig. 1). The corresponding Ca/P values, calculated using the above formula, are presented in Fig. 2. These results are well consistent with those of chemical analyses [5,6,16,17]. For example, the time required for forming pure-phase HA at 25 °C (a somewhat higher temperature than 21 °C in this study) and under other comparable synthesis variables in a recent comprehensive study of nitrous synthesis was 25 h [6].

## IV. Conclusions

X-ray powder diffraction can be a simple and fast method for monitoring the extent of crystallization and measuring the Ca/P ratio in the precipitates at any stage of HA wet synthesis.

The obtained quantitative dependences of the phase composition and (or) Ca/P ratio in the precipitates on the synthesis time can effectively be applied in processing calcium phosphate ceramics based on HA of any desired composition.

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