COST Action MP1301
"New generation biomimetic and customized implants for bone engineering" (NewGen)

Short term scientific mission (STSM)
"New biomimetic amorphous calcium phosphate biomaterials: structure and thermal properties"

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SCIENTIFIC REPORT: A SUMMARY

1. Introduction

Every year there is increasing number of patients that have bone injuries and bone diseases [1][2], therefore research on biomaterials for bone repair, replacement and medical treatment continues. Important role is dedicated to calcium phosphates (CaP) [3] and CaP containing composite [4] and hybrid [5] materials for bone regeneration etc. Current STSM was dedicated to synthesis and characterization of new amorphous and low crystalline calcium phosphate nanoparticles. These nanoparticles could be used as a base for preparation of calcium phosphates with controlled crystallinity degree.

Aim of the STSM was to prepare biomimetic amorphous and low crystalline CaP nanoparticles and to characterize their structure and thermal properties. Also technological features (pH, drying method) were compared in order to shorten drying time and reduce costs while preserving the amorphous structure and properties of the product.

Tasks:

1) Prepare CaPs at different pH values with novel synthesis method;
2) Characterize thermal properties of obtained CaPs;
3) Characterize thermally treated CaPs;
3) Determine particle size of CaP nanoparticles after synthesis.

Thermal and structural studies will complete characterization of CaP nanoparticles and allow to predict material behaviour *in vitro*, *in vivo* and in long term. These data will help in preparation of biomaterials with specific properties, in understanding of biomineralization processes (formation of natural CaP in bone, calcification phenomena both in human and animal models).

2. Materials and Methods

2.1. Synthesis of calcium phosphate nanoparticles

Calcium phosphate (CaP) nanoparticles with variable crystallinity degree were prepared by reprecipitation process from hydroxyapatite (HAp) suspension. Technological process in brief, HAp synthesized at R.Cimdins Riga Biomaterials Innovation and Development Centre of Riga Technical University was used to prepare initial suspension. HAp was prepared by wet precipitation process from $Ca(OH)_2$ and H_3PO_4 at end pH 8.8 [6]. HAp was dissolved in hydrochloric acid and stirred for 30 min. Ion species were induced to rapidly reorganize into amorphous or low crystalline solids by

fast pH change of the solution by addition of strong base. End pH of the synthesis was adjusted with ammonia solution. Formed precipitates were separated and washed several times with deionized water-ethanol solutions. Gel-like precipitates were dried either in freeze dryer or hot air oven.

2.2. Characterization methodology

Analysed materials: Amorphous or low crystalline calcium phosphates. The variables are – pH of the end of the synthesis and drying method (oven or freeze drying).

Reference materials: commercial hydroxyapatite nanopowder n-HAp, ≥97% (Sigma Aldrich) and hydroxyapatite R-HAp powder prepared at Riga Technical University by wet precipitation method.

Equipment:

At Aalto University, Finland:

- Simultaneous TG-DTA/DSC Apparatus STA449C Jupiter® (Netzsch, Germany) for thermal properties measurement;
- Zetasizer (Malvern, UK) for particle size distribution analysis. Measuring range 0.6-6000 nm.

At Riga Technical University, Latvia:

- X-Ray diffractometer X'pert Pro (Panalytical, the Netherlands);
- FT-IR spectrometer 800 Scimitar Series (Varian, USA) with ATR unit;
- Specific surface analysis with N_2 adsorption system Quadrasorb SI (Quantachrome Instruments, USA).

Sample preparation for DSC-TGA.

Small amount of sample were put in alumina crucibles with lids. Lid had a hole in it. Sample mass for all measurements was approximately 20 mg for oven dried and 5 mg for freeze dried samples. Apparatus was purged with argon to avoid sample-gas interaction. Gas flow was 10 mL/min. Heating rate was 10°C/min and the measurement was conducted from 30 to 1200°C. As a reference identical empty crucible with lid was used. All samples and their characteristics for DSC-TGA measurements are summarized in Table 1. A baseline measurement with empty reference and sample crucibles was run as well.

Table 1.

Summary of samples analysed with DSC-TGA

No.	Sample abbreviation	pH at the end of the synthesis	Drying method	Sample type
1.	ACP-8-O	8	Oven (80°C)	Fine powder
2.	ACP-9-O	9		
3.	ACP-10-O	10		
4.	ACP-11-O	11		
5.	ACP-8-F	8	Freeze drying (72 h)	
6.	ACP-9-F	9		
7.	ACP-10-F	10		
8.	ACP-11-F	11		
9.	Reference HAp, RTU	8-9	Oven (80°C)	
10.	Reference nano-HAp, Sigma Aldrich	-	-	

Sample preparation for particle size analysis. For particle size distribution analysis at early stages of calcium phosphate precipitation Zetasizer (Malvern) was used (Table 2). The synthesis was performed and 15 min after the CaP precipitation the PSD measurement was done. The sample was taken in following way – a drop of synthesis solution was highly diluted to avoid obscuration in the instrument. 1 mL of diluted sample was transferred to measuring cuvette. The measurements

were conducted in room temperature. It is important that no sedimentation or aggregation of particles occurs.

Table 2.

Summary of samples analysed with PSD analyser Zetasizer

No.	Sample	pH at the end of the synthesis	Sample type
1.	ACP-8	8	Very diluted synthesis suspension
2.	ACP-9	9	
3.	ACP-10	10	
4.	ACP-11	11	

Equation for crystallinity degree X_c calculation for samples containing *only HAp*:

$X_c\,\% = (1 - \upsilon_{(112/300)}/I_{300}) \times 100\%$,

where $\upsilon_{(112/300)}$ – the intensity of hollow between (112) and (300) difraction peaks of HAp and I_{300} is the intensity of the (300) diffraction peak of HAp [7].

3. Results

3.1. Powder X-Ray diffraction.

In Fig. 1 X-Ray diffraction (XRD) patterns of both oven-dried (Ov) and freeze-dried (FrD) calcium phosphates after synthesis are shown.

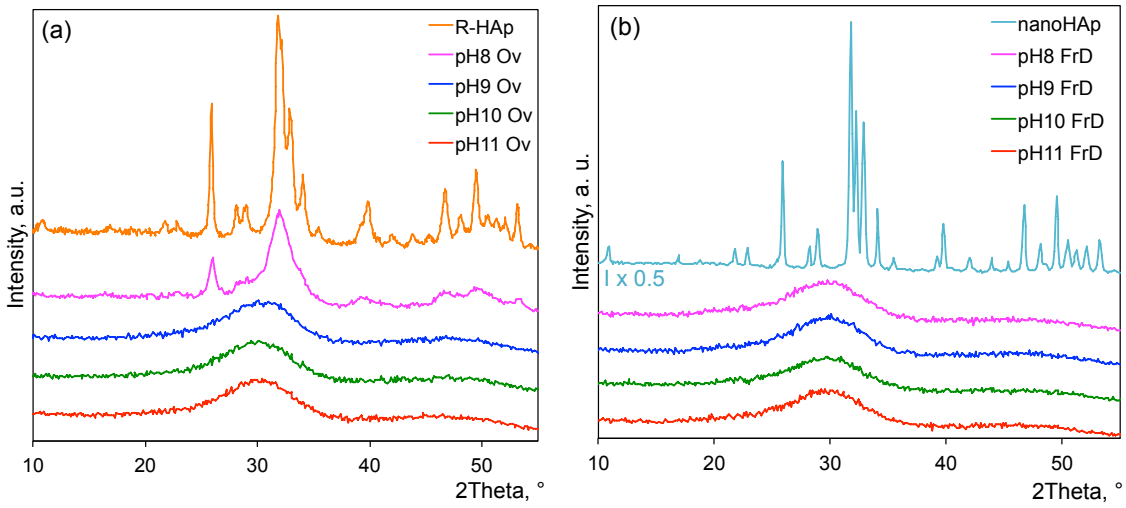

Fig. 1. X-Ray diffraction patterns of oven dried (a) and freeze dried (b) calcium phosphate nanoparticles. R-Hap and nanoHAp are added as a reference.

Several characteristics from XRD patterns were calculated for samples after DSC-TGA measurement as shown on Table 3. For X-ray amorphous samples crystallinity degree was assumed to be zero.

Table 3.

Calculated characteristics of CaPs after DSC analysis from XRD

Sample	Drying method	Quantitative phase composition, wt%	Ca/P ratio	Crystallinity degree	
				before DSC-TGA	*after* DSC-TGA, 1200°C
ACP-pH8	Oven	β-TCP	1.50	*	*
ACP-pH9		β-TCP	1.50	0	*
ACP-pH10		β-TCP (77%), HAp (23%)	1.54	0	*
ACP-pH11		β-TCP (87%), HAp (13%)	1.52	0	*

ACP-pH8	Freeze drying	β-TCP	1.50	0	*
ACP-pH9		β-TCP	1.50	0	*
ACP-pH10		β-TCP (79%), HAp (21%)	1.53	0	*
ACP-pH11		β-TCP (87%), HAp (13%)	1.52	0	*
R-HAp	Oven	HAp	1.67	31	95
nano-HAp	n.a.	HAp	1.67	87	95

* - measurements in progress

3.2. FT-IR spectroscopy

For complete structure description FT-IR spectra of as-synthesized and in DSC-TGA analyzed CaPs were taken (Fig. 2 and Fig. 3). As synthesized powders have broad absorption bands without sharp peaks while heat-treated (DSC-TGA) and reference samples show sharp and pronounced absorption bands characteristic to crystalline materials.

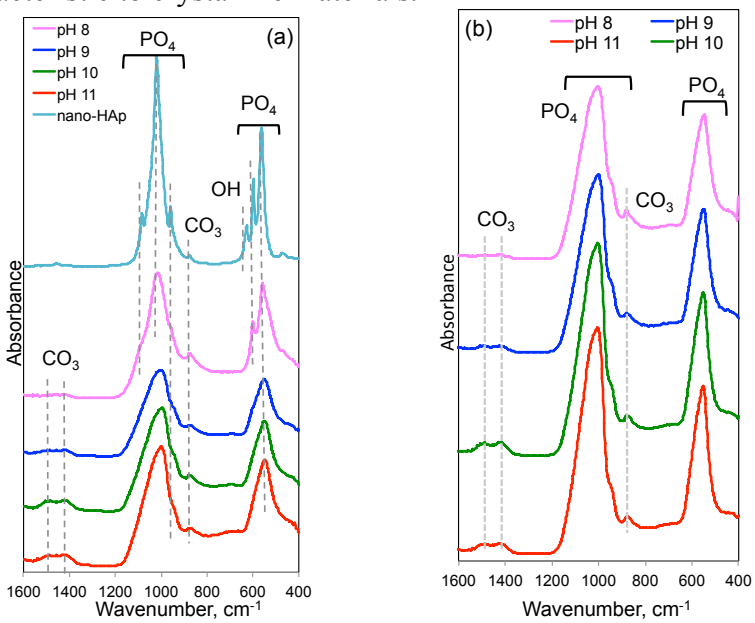

Fig. 2. FT-IR ATR spectra of oven dried (a) and freeze dried (b) calcium phosphate nanoparticles after synthesis and nano-HAp.

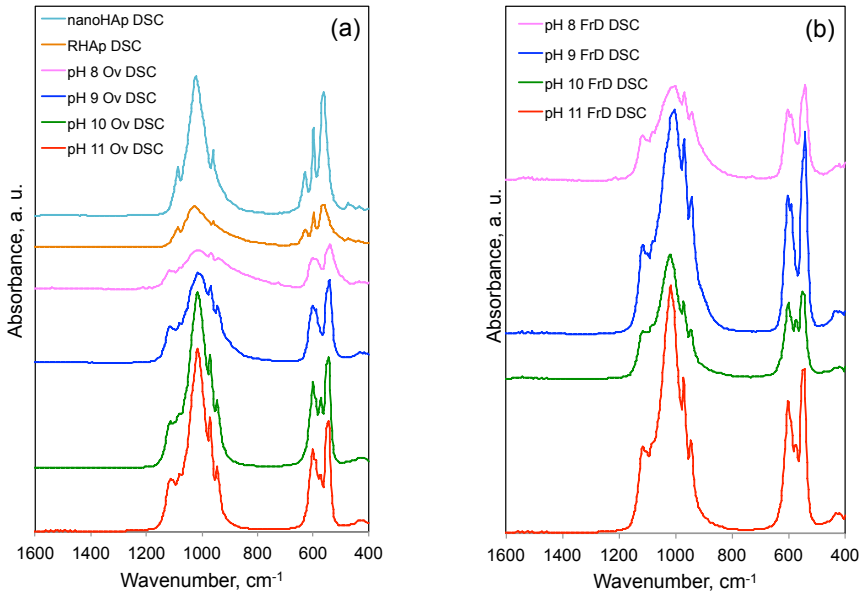

Fig. 3. FT-IR ATR spectra of oven dried (a) and freeze-dried (b) calcium phosphate nanoparticles after DSC measurement (1200°C).

3.3. Specific surface area

Obtained as synthesized materials have high SSA (126-154 m^2/g), that is 32-62% higher than the starting material R-HAp. Nano-HAp has rather small SSA (12 m^2/g), because of possible heat treatment. Particle sizes calculated after BET are in the range 14-17 nm.

3.4. Thermal analysis

3.4.1. Thermogravimetry (TGA)

TGA curves of both oven-dried and freeze-dried calcium phosphates are shown on Fig. 4. Most of the volatiles (residual water and ammonia) are lost till 400°C. R-HAp and nano-HAp have the smallest mass loss – 4.8 % and 1.7 % (till 1200°C), respectively.

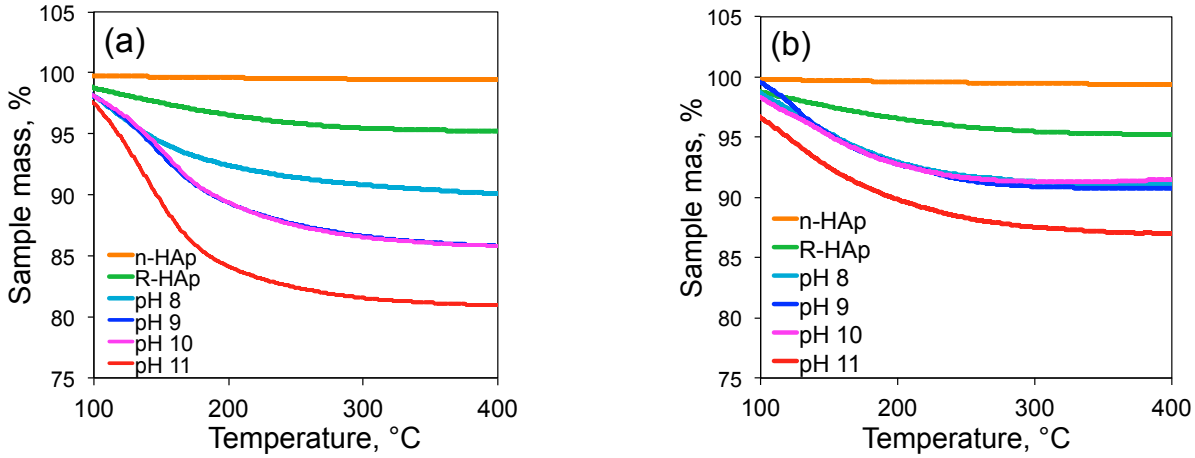

Fig. 4. TGA curves of oven dried (a) and freeze dried (b) calcium phosphates. Region from 100-400°C is shown.

3.4.2. Differential Scanning Calorimetry.

Observed exothermic effects of CaPs during DSC analysis are shown on Fig.5. The thermal effect is crystallization of amorphous phase into ordered structure. Thermal effect is not associated with simultaneous mass loss when compared with TGA curves. There is no direct trend between peak parameters and synthesis pH or amount of lost mass.

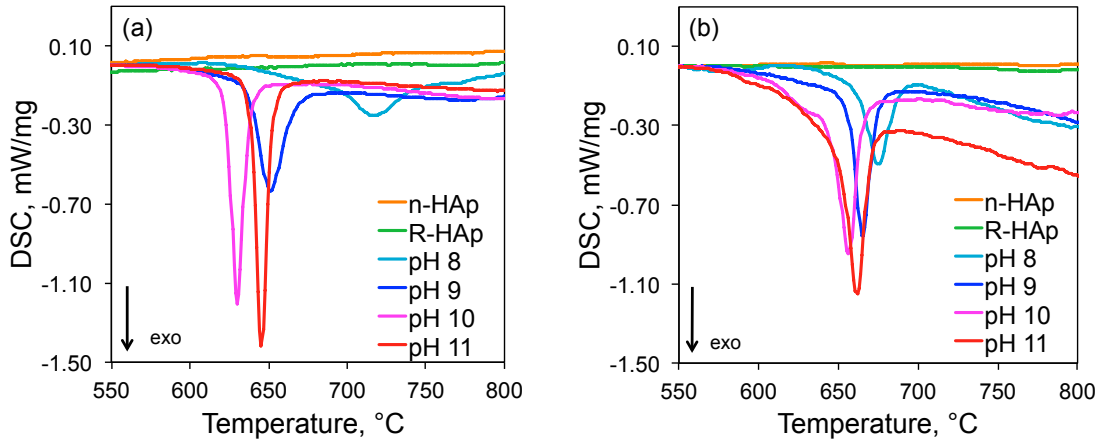

Fig. 5. DSC curves of oven dried (a) and freeze-dried (b) calcium phosphates; 550-800°C region where crystallization occurs is shown.

3.5. Particle size analysis

63 various experiments were carried out to determine particle size distribution of as synthesized CaP nanoparticles with Malvern Zetasizer. Unfortunately very strong nanoparticle agglomeration and sedimentation of these agglomerates did not allow performing the measurement within apparatus size range limit (1-10 nm). Ultra-sonication of samples did not break the aggregates.

4. Conclusions

- Biomimetic amorphous and low crystalline calcium phosphate (CaP) nanoparticles with high specific surface were prepared by a novel synthesis method.
- Obtained CaPs had different Ca/P ratios depending on synthesis pH, therefore properties (solubility, sinterability etc) could be adjustable to application specific needs.
- Synthesis pH and the amount of free OH^- have some effect on structure of oven dried CaPs.
- Amorphous and low crystalline CaPs transforms into β-TCP and HAp phases upon heating, with onset of the process over 600-650°C.
- Oven drying produced small differences on as-synthesized CaP structure, but freeze-drying did not – all samples were amorphous.

The combined use of the unique equipment and facilities in host institution (AALTO) has allowed the applicant to study and find scientific evidences on the CaP transformations vs. synthesis route and parameters. Such studies were not possibly to make in the applicant institutions alone.

STSM Applicant and Host together will write a scientific paper on basis of results obtained at STSM to foster cooperation between organizations and popularize COST Action MP1301.

References

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