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Calcium metal to synthesize amorphous or cryptocrystalline calcium phosphates ☆

A. Cuneyt Tas

Department of Biomedical Engineering, Yeditepe University, Istanbul 34755, Turkey

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ABSTRACT

Metallic calcium was never used before as the calcium source in synthesizing bioceramics. Amorphous calcium phosphate (ACP) powders were synthesized at room temperature, in synthetic mineralization solutions which contained Na^+, K^+, Mg^{2+}, Cl^-, HCO_3^- and HPO_4^{2-} ions at concentrations similar to those found in human blood plasma, by using calcium (Ca) metal as the only calcium source. The experimental conditions leading to the formation of PCA (cryptocrystalline or poorly crystallized apatite) or $CaCO_3$ powders were also determined when using metallic Ca in aqueous synthesis in the mineralization solutions. The formation of calcium phosphate (CaP) in synthesis solutions was immediately initiated by the addition of calcium metal granules (or shots), at appropriate amounts, into the solutions while the solutions were being continuously stirred in glass bottles at room temperature (22 ± 1 °C). The synthesis reactions were reaching completion in less than 30 min with the final solution pH values ranging from 9 to 12, without a necessity for any external pH adjustment in the form of any strong base (such as NH_4OH, LiOH, NaOH or KOH) additions. ACP or PCA powders are useful for dentin and enamel re-mineralization applications or orthopedic (bone) defect-filling applications.

1. Introduction

The systematic synthesis and characterization of poorly-crystallized (i.e., cryptocrystalline) apatite (PCA) powders in deionized (i.e., free of Na^+, K^+, Mg^{2+}, Cl^- and HCO_3^- ions of blood plasma) water solutions containing dissolved calcium nitrate tetrahdyrate ($Ca(NO_3)_2 \cdot 4H_2O$) and diammonium hydrogen phosphate ($(NH_4)_2HPO_4$) were initiated in the early 50s by Hayek and co-workers [1–3]. The work of Hayek et al. [1–3] taught us to raise the pH values of such cryptocrystalline apatite synthesis solutions to around 10.5–11 by the addition of ammonium hydroxide (NH_4OH). The method originally developed by Hayek et al. [1–3] to produce cryptocrystalline nanoparticles of calcium phosphate (CaP) was then adopted and popularized by Jarcho et al. [4]. Currently, the above-mentioned Hayek method of synthesizing cryptocrystalline apatitic CaP powders is one of the most preferred.

Posner and co-workers [5–7] were among the first, in the mid 50s, to realize that the mineral of natural hard tissues consisted of non-stoichiometric pseudoapatites. Posner et al. [5] envisaged in as early as 1954 that the carbonate (CO_3)- and foreign cation-free pseudoapatites can be represented by the formula of $Ca_{10-x}H_{2x}(PO_4)_6(OH)_2$, where the value of x would range from zero for stoichiometric hydroxyapatite (HA) to two for an apatite with a Ca/P molar ratio of 1.333. One shall here note the similarity between that pseudoapatite of Ca/P ratio of 1.333 mentioned by Posner [5] and the compound known as octacalcium phosphate (OCP, $Ca_8H_2(PO_4)_6 \cdot 5H_2O$ or more appropriately denoted as $Ca_8(HPO_4)_2(PO_4)_4 \cdot 5H_2O$). Posner et al. [8] later showed experimentally that the mineral of the rat bones did exhibit a very strong age-dependent (from 1 to 40 days of age) variation in their Ca/P molar ratios, i.e., the younger the rat the lower the Ca/P ratio of its bones, the very young rats having a Ca/P ratio over the range of 1.15 to 1.34, in agreement with the much earlier work of Burns and Henderson [9].

Posner and co-workers [10] have been the first to describe how to prepare synthetic amorphous calcium phosphate (ACP) powders by using $CaCl_2$- and $(NH_4)_2HPO_4$-containing distilled water solutions (i.e., not containing biologically essential ions such as Na^+, Mg^{2+}, K^+ or HCO_3^-) whose pH values were raised to around 11 by NH_4OH additions.

Betts and Posner [11,12] postulated that ACP actually consisted of roughly spherical clusters (also called as *Posner clusters*) close to 1 nm in diameter, with a Ca/P molar ratio of 1.5 and the formula of $Ca_9(PO_4)_6$, which were free of water. Synthetic ACP, according to Posner et al. [11,12], consisted of roughly spherical $Ca_9(PO_4)_6$ clusters, which formed in water and were then aggregated randomly to produce the larger spherical particles of ACP with the inter-cluster space being filled with water. A similar aggregation process was however described previously, in 1957, by Glimcher et al. [13] in relation to mineralization in the collagen-hydroxyapatite system.

ACP, when in contact with an aqueous solution, is known to exhibit the unique ability to first nucleate OCP-like nanosize crystallites on

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E-mail addresses: c_tas@hotmail.com, actas@yeditepe.edu.tr.
URL: http://www.cuneyttas.com.

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the surfaces of its particles, which would then rapidly mature into apatitic calcium phosphate [10,12]. This property of ACP powders was successfully exploited to prepare injectable orthopedic cements [14,15]. Posner and his co-workers were also the first to study the interaction of casein micelles of bovine milk with ACP powders [16], and this apparently led to the development of ACP-casein phosphopeptide (CPP) [17] complexes for dental remineralization applications.

Since the early studies of Hayek [1–3] and Posner [5,10–13], to the best of our knowledge, the ACP and PCA-related literature [18–35] did not contain any novel approaches to the synthesis of ACP powders, i.e., meaning the calcium source employed in the synthesis processes was always selected from the Ca-chloride, Ca-nitrate and Ca-acetate salt group, and the pH values of the synthesis solutions were raised to the basic range (pH ~11) by the addition of strong bases such as NH_4OH, NaOH or KOH.

The current study was originated by the following questions:

(i) Could it ever be possible to synthesize CaP powders (either ACP or PCA) in aqueous solutions totally free of nitrate (NO_3), acetate (CH_3COO) or ammonium (NH_4) ions which are not shown to be present in biological bone or tooth formation processes?
(ii) Could it be possible to synthesize ACP or PCA powders by using aqueous solutions having the pH values from 9 to 12 (which was underlined by the early works of Hayek and Posner as a necessity) without even using the smallest aliquot of a strong base such as NH_4OH, NaOH, KOH or LiOH?
(iii) Could it be possible to simulate the concentrations of inorganic ions present in human blood plasma in the synthesis solutions while strictly maintaining the conditions set by the above two questions?

In CaP synthesis, nitrate or acetate ions would be introduced into the synthesis solutions by the use of calcium nitrate tetrahydrate or calcium acetate monohydrate as the calcium source. We considered that if we were using Ca metal as the only calcium source, then that would totally eliminate any nitrate or acetate ions. Layrolle et al. [27] used Ca metal shots only to produce calcium diethoxide by reacting them with ethanol, and they did not see the Ca metal shots as a possible starting material to be quite useful in ACP or PCA synthesis in aqueous solutions. It is common knowledge [36] that Ca metal is produced by electrolysis of a molten bath of calcium chloride salt, and the produced Ca metal granules react with distilled water to raise its pH under a slow evolution of H_2 gas (i.e., in situ deprotonation). We considered that the use of Ca metal as the calcium source in ACP or PCA synthesis might have eliminated the need for using of any strong bases in raising the solution pH to the levels given in the works of Hayek [1–3] and Posner [5,10–13].

This study, to the best of our knowledge, is the first one to use Ca metal (but not salts such as Ca-chloride, Ca-nitrate or Ca-acetate) in synthesizing calcium phosphates. This study also compared the use of Ca metal (as the Ca source) to using the above-mentioned calcium salts. This study would also be the first one to synthesize ACP (or PCA) powders in synthetic mineralization solutions developed hereby to mimick the inorganic ion concentrations of human blood plasma. The human body does not use deionized or distilled water in synthesizing the mineralized portion of bone and teeth.

2. Materials and methods

2.1. Materials

Sodium chloride (NaCl; Catalog No: 1.06404, Merck KGaA, Darmstadt, Germany), potassium chloride (KCl; Cat. No: 1.210517, Merck), magnesium chloride hexahydrate ($MgCl_2{\cdot}6H_2O$; Cat. No: 459331, Carlo Erba Reagenti, Milano, Italy), sodium bicarbonate ($NaHCO_3$; Cat. No: 1.06329, Merck), and disodium hydrogen phosphate anhydrous (Na_2HPO_4; Cat. No: 1.06586, Merck) were used in solution preparation.

Calcium metal (Ca; spherical granular, 2–4 mm in diameter; Cat. No: 1.02053, Merck), calcium chloride dihydrate ($CaCl_2{\cdot}2H_2O$; Cat. No: 1.02382, Merck), calcium nitrate tetrahydrate ($Ca(NO_3)_2{\cdot}4H_2O$; Cat. No: 1.02121, Merck), calcium acetate monohydrate ($Ca(CH_3CO_2)_2{\cdot}H_2O$; Cat. No: 402850, Sigma-Aldrich), and calcium hydroxide ($Ca(OH)_2$; Cat. No: 1.02110, Merck) were separately tested as the sources of calcium.

In some experiments diammonium hydrogen phosphate ($(NH_4)_2HPO_4$; Cat. No: 1.01207, Merck) was tested as the source of phosphorus instead of Na_2HPO_4. Finally, ammonium hydrogen carbonate (NH_4HCO_3, Cat. No: 1.01131, Merck) was also tested to replace $NaHCO_3$ in some experiments.

2.2. Solution preparation and synthesis

The solutions were prepared in 500 mL-capacity Pyrex™ glass bottles (Fisher Scientific, Cat. No: FB800500). The bottles were first cleaned by washing with 5 vol.% HCl, followed by rinsing with an ample amount of doubly-distilled water, and overnight drying at 90 °C. Five hundred milliliter of doubly-distilled water was first placed into the bottles at room temperature (RT, 22±1 °C). A Teflon®-coated (25 mm long, 5 mm in diameter) rod-shaped magnetic stirrer was then placed into the experiment bottle. All of the synthesis experiments were performed on a magnetic stir-plate and the stirring rate for all experiments was kept constant at 750 rpm. The carefully weighed chemicals were added, one by one, to the bottle, under constant stirring of the solution inside. The next chemical was not added prior to the complete dissolution of the previous one. Table 1 shows the procedure of preparing the synthesis/mineralization solutions (MS) in 500 mL distilled water (not boiled prior to use to remove any possible HCO_3^-). The chemicals were added to water in the order given. Table 1 offered three choices of solution preparation to the reader; the first one would lead to preparing a solution with 10 mM HPO_4^{2-}, whereas the third would result in a solution with 1 mM HPO_4^{2-}. All solutions shown in Table 1 were fully transparent at the time of preparation, and thus they were ready for the addition of the pre-weighed amount of Ca metal (or calcium chloride, calcium acetate monohydrate or calcium nitrate tetrahydrate in a limited number of experiments).

To further clarify the solution preparation technique described in Table 1; one first adds KCl to 500 mL of water, dissolves it, then performs the respective additions of $MgCl_2{\cdot}6H_2O$, NaCl and $NaHCO_3$. At that moment, the solutions contain 5 mM K^+, 1.5 mM Mg^{2+}, 103 mM Cl^-, and 27 mM HCO_3^-. These ion concentrations are identical with those of the blood plasma. If one were then adding 0.7098 g of Na_2HPO_4, the solution would have a total Na^+ ion concentration equal to 142 mM. This concentration of Na^+ is exactly that of the blood plasma. The solution thus obtained according to the choice-1 of Table 1 was able to match the Na^+, K^+, Mg^{2+}, HCO_3^-, Cl^- concentrations of the blood plasma, but will possess 10 times the HPO_4^{2-} concentration of plasma. However, the solution of choice-3 (of

Table 1
Preparation of mineralization solutions (MS) *500 mL H_2O basis.*

Chemical	*g*	*mM* cation	*mM* anion
KCl	0.1865	5 K^+	5 Cl^-
$MgCl_2{\cdot}6H_2O$	0.1525	1.5 Mg^{2+}	3 Cl^-
NaCl	2.7760	95 Na^+	95 Cl^-
$NaHCO_3$	1.1341	27 Na^+	27 HCO_3^-
Choices:			
(*1*) Na_2HPO_4	0.7098	20 Na^+	10 HPO_4^{2-}
(*2*) Na_2HPO_4	0.3549	10 Na^+	5 HPO_4^{2-}
(*3*) Na_2HPO_4	0.0710	2 Na^+	1 HPO_4^{2-}

Table 1) will have the identical HPO_4^{2-} concentration with that of blood plasma.

If one were using $CaCl_2 \cdot 2H_2O$ as the calcium source (instead of Ca metal), it would not be possible to maintain the proper Cl^- ion concentration in the solution, i.e., it would have been in excess of 103 mM. Blood plasma contains exactly 103 mM Cl^-. If one were using $Ca(NO_3)_2 \cdot 4H_2O$ as the calcium source, then the synthesis medium would have contained nitrate ions, which are not present in the blood plasma. The same applies to the use of Ca-acetate, as well.

Powder synthesis began instantly by the addition of prescribed amount of calcium metal granules into the mineralization solutions stirred at 750 rpm. Reactions were continued for 25 min at RT (22 ± 1 °C). pH values were recorded (pH meter, Model: S40, Mettler-Toledo, w/ combined pH-temperature electrode), at every 30 s, starting from the moment of adding Ca metal into the solutions. At the end of 25 min of stirring, the formed solids were immediately and quickly filtered out of their mother liquors by using a Whatman No. 2 filter paper *via* a Buechner funnel apparatus, backed up with a mechanical vacuum pump. The solid residues were washed with 750 mL of distilled water and then dried on watch-glasses at RT for 48 h in an air-ventilated drying cabin. In the duplicate experiments, samples were synthesized once more as described above, but then left in the solutions overnight (i.e., at least 17 h), in the bottles, at RT. The pH values of the solutions were measured once again after that long period of RT aging and exactly the same values were found with those measured after only 25 min of reaction.

2.3. Sample characterization

Prior to powder X-ray diffraction (XRD) and Fourier-transform infrared spectroscopy (FTIR) analyses, the dried samples were ground, manually, in an agate mortar by using an agate pestle. XRD runs were performed (Advance D8, Bruker, Karlsruhe, Germany) in the step scan mode, with the step size of 0.02° and preset time of 5 s. The powder diffractometer was equipped with a Cu tube and operated at 40 kV and 40 mA. XRD samples were prepared by gently packing the powders into the sample holder cavity of around 1 mm-deep. FTIR samples were mixed with KBr powders at the ratio of 1 mg sample-to-250 mg KBr in an agate mortar. FTIR pellets of 13 mm diameter were pressed at 10 tons. FTIR data were collected (Spectrum One, PerkinElmer, Waltham, MA) by using 256 scans. Scanning electron microscopy (Vega-3, Tescan, A.S., Brno, Czech Republic) samples were not ground and the small sample chunks were sputter-coated with a thin gold layer before imaging.

3. Results and discussion

Until this study, the researchers working in CaP-based biomaterial synthesis have chosen either one of the following as their calcium source; calcium chloride (anhydrous or dihydrate), calcium nitrate (tetrahydrate), calcium acetate (monohydrate), calcium carbonate or calcium hydroxide. The former three of these have significant solubility even in cold water, but the latter two had much lower solubilities in comparison to the former. The major drawback associated with the use of calcium nitrate or calcium acetate was that the synthesized phases would be poisoned by the residual nitrate or acetate ions, as it was experimentally proven by Ivanova et al. [37].

One of the novelties of this study is that it used metallic calcium as the calcium source. Metallic calcium presented clear advantages: (*i*) it did not bring into the synthesis solutions any foreign or spectator anions, such as nitrates, chlorides or acetates, and (*ii*) it caused in situ deprotonation of the aqueous synthesis media resulting in a smooth and rapid pH increase (as shown below), totally eliminating the need for base (NaOH, KOH, LiOH, NH_4OH, etc.) additions to maintain the synthesis pH above neutral. These two points further define the novelty of this study. The necessity of maintaining the solution pH much above the neutral during the synthesis of either ACP (amorphous calcium phosphate) or PCA (poorly-crystallized, cryptocrystalline apatitic calcium phosphate) was well-proven throughout the previous work of Hayek [1–3], Posner [5–7] and Rey [14,15]. Rey et al. [15], for instance, prepared their ACP powders by mixing an aqueous solution of Ca-nitrate with another solution containing disodium hydrogen

Table 2
Details of selected experiments.

Experiment	P source	Ca source	CO_3 source	P (mM)	Ca (mM)	CO_3 (mM)	Final pH	Phases/XRD	Medium
1	–	Ca	–	–	25	–	12.6	$Ca(OH)_2 + CaCO_3$	H_2O
2	–	Ca	$NaHCO_3$	–	25	27	9.9	$CaCO_3$	5 KCl + 1.5 $MgCl_2$
3	–	Ca	$NaHCO_3$	–	25	27	9.9	$CaCO_3$	H_2O
4	–	Ca	$NaHCO_3$	–	25	27	12.3	$CaCO_3$	95 NaCl
5	–	Ca	$NaHCO_3$	–	25	27	12.3	$CaCO_3$	MS
6	Na_2HPO_4	Ca	–	10	25	–	12.3	PCA + $CaCO_3$	H_2O
7	Na_2HPO_4	Ca	–	10	16.667	–	12.2	PCA	MS *w/o* HCO_3
8	Na_2HPO_4	Ca	–	10	25	–	12.4	PCA	MS *w/o* HCO_3
9	$(NH_4)_2HPO_4$	Ca	–	10	16.667	–	11.3	ACP	MS *w/o* HCO_3
10	$(NH_4)_2HPO_4$	Ca	–	10	25	–	12.0	PCA	MS *w/o* HCO_3
11	Na_2HPO_4	$CaCl_2 \cdot 2H_2O$	–	10	25	–	5.9	DCPD + PCA	H_2O
12	$(NH_4)_2HPO_4$	$CaCl_2 \cdot 2H_2O$	–	10	16.667	–	6.5	DCPD	MS *w/o* HCO_3
13	$(NH_4)_2HPO_4$	$CaCl_2 \cdot 2H_2O$	–	10	25	–	6.5	DCPD + PCA	MS *w/o* HCO_3
14	$(NH_4)_2HPO_4$	$CaCl_2 \cdot 2H_2O$	–	10	50	–	5.7	DCPD + PCA	MS *w/o* HCO_3
15	$(NH_4)_2HPO_4$	$CaCl_2 \cdot 2H_2O$	–	10	16.667	–	6.1	DCPD + PCA	H_2O
16	Na_2HPO_4	Ca	$NaHCO_3$	1	2.5	27	9.2	ACP	MS
17	Na_2HPO_4	Ca	$NaHCO_3$	5	12.5	27	10.3	ACP	MS
18	Na_2HPO_4	Ca	$NaHCO_3$	10	25	27	12.0	ACP + $CaCO_3$	MS
19	Na_2HPO_4	Ca	$NaHCO_3$	10	25	27	9.0	No ppt^s	H_2O
20	$(NH_4)_2HPO_4$	Ca	$NaHCO_3$	10	25	27	10.4	ACP + $CaCO_3$	MS
21	Na_2HPO_4	Ca	NH_4HCO_3	10	25	27	10.1	ACP + $CaCO_3$	MS
22	$(NH_4)_2HPO_4$	Ca	NH_4HCO_3	6.667	16.667	27	9.4	ACP	MS
23	$(NH_4)_2HPO_4$	Ca	NH_4HCO_3	10	16.667	27	9.3	ACP	MS
24	$(NH_4)_2HPO_4$	Ca	NH_4HCO_3	10	25	27	9.5	ACP	MS
25	Na_2HPO_4	$CaCl_2 \cdot 2H_2O$	$NaHCO_3$	10	25	27	7.0	PCA	MS
26	Na_2HPO_4	$CaCl_2 \cdot 2H_2O$	$NaHCO_3$	10	25	27	7.0	PCA	H_2O
27	$(NH_4)_2HPO_4$	$CaCl_2 \cdot 2H_2O$	NH_4HCO_3	10	25	27	7.0	PCA	MS
28	Na_2HPO_4	Ca-acetate	$NaHCO_3$	10	25	27	7.0	PCA	MS
29	Na_2HPO_4	Ca-nitrate	$NaHCO_3$	10	25	27	7.0	PCA	MS
30	Na_2HPO_4	$Ca(OH)_2$	$NaHCO_3$	10	25	27	11.7	ACP + $CaCO_3$	MS

phosphate, 1 M NaOH and 0.3 M $NaHCO_3$. The second solution [15] had a very high Na^+ concentration (i.e., 1.3 M). In stark contrast, the Na^+ concentration of the solutions of the current study was kept constant at only 142 mM, which is the sodium concentration of human blood plasma. Reaching pH values in the vicinity of 9 to 12 by using such low concentrations of Na^+ is another advantage of the use of Ca metal.

3.1. Synthesizing $CaCO_3$ by using metallic Ca

The starting point of this study was to find an answer to the following simple question: what happens if one adds 25 mM (i.e., 10 times the calcium concentration of blood plasma) of Ca metal granules (*i*) into water, (*ii*) into saline (NaCl-, KCl- and/or $MgCl_2 \cdot 6H_2O$-containing) water, or (*iii*) into carbonated (HCO_3-containing, but no chlorides) water, and then stir the granules at RT in these solutions for only a finite time, such as 25 min? The experiments detailed in Table 2 summarized the design of this study.

Calcium granules stirred in doubly-distilled water for 25 min (with a rise in solution pH to around 12) were not dissolved (Experiment-1 of Table 2), they rather seemed to be rapidly covered with a white layer consisting of a biphasic mixture of $Ca(OH)_2$ and $CaCO_3$, as determined by their XRD data given in Fig. 1a. XRD data, only in this case, were collected from the *as-recovered* granules, without attempting to crush them. One can further speculate here that the incident X-rays would not be able to pass through the hydroxide-carbonate layer formed on the granules to reach their still metallic cores.

25 mM of calcium granules stirred for 25 min in an aqueous solution containing only 5 mM K^+, 1.5 mM Mg^{2+} and 27 mM HCO_3^- did not totally dissolve. The Cl^- ion concentration of this solution was equal to 8 mM (Experiment-2 of Table 2), but the K^+ and Mg^{2+} concentrations were equal to that of blood plasma. Ca granules did not dissolve in distilled water (Exp-1), and they also did not totally dissolve in a solution containing 8 mM Cl^- and 27 mM HCO_3^- (Exp-2). In these two experiments, the rapid formation of a biphasic layer of $Ca(OH)_2$ (major phase) and $CaCO_3$ (minor phase) on the surfaces of the Ca metal granule was observed.

In Exp-3 (Table 2), 25 mM calcium granules were stirred in distilled water containing only 27 mM Na^+ and 27 mM HCO_3^- (no Cl^-). Granules did not dissolve. Very small amounts of solution precipitates formed in experiments 2 and 3 proved, by XRD and FTIR, to be single-phase $CaCO_3$.

Cl^- concentration was increased to 95 mM in Exp-4. 25 mM of calcium granules stirred in an aqueous solution (Exp-4) containing 122 (=95+27) mM Na^+, 95 mM Cl^-, and 27 mM HCO_3^- were dissolved completely and produced quite a significant amount of $CaCO_3$ precipitate in the solution in 25 min. We have thus experimentally determined that there seemed to be a close relationship between the complete dissolution of the Ca metal granules and Cl^- concentration of the solution into which they were placed. Ca metal granules added into aqueous solutions caused the evolution of H_2 gas (i.e., in situ deprotonation [36]), but that gas evolution slowed down by the formation of a hydroxide layer on the granule surfaces at low Cl^- concentrations. Moreover, since the granule size used in this study was 2 to 4 mm, that gas evolution was not so fierce.

We speculate that in solutions containing increased amounts of Cl^-, H_2 gas evolving at the granule surfaces was creating a microenvironment

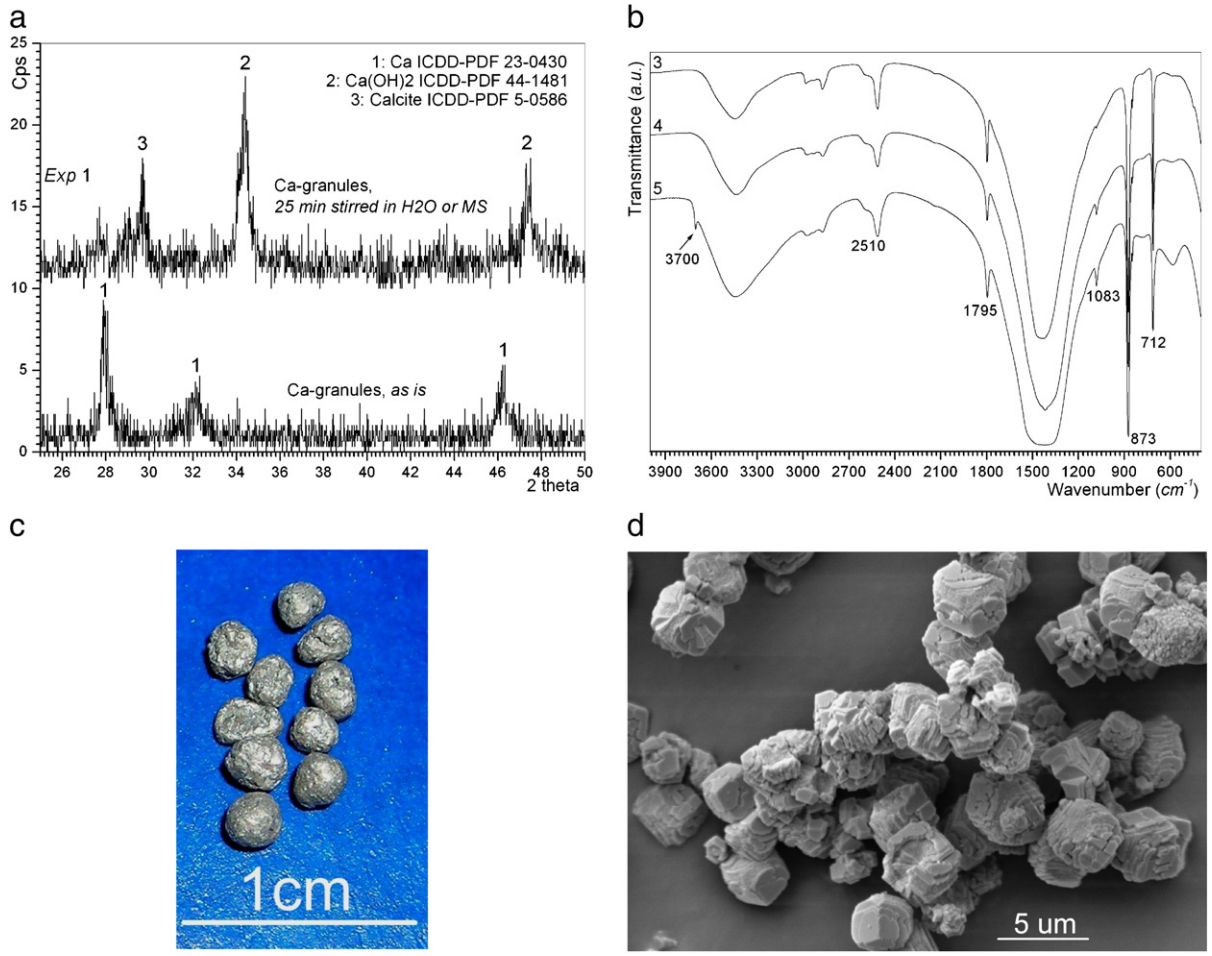

Fig. 1. a. XRD traces of as-received Ca granules (bottom) and Ca granules stirred in H_2O or MS (top, Exp. 1). b. FTIR traces of the samples of experiments 3, 4, and 5. c. Macrophotograph of as-received Ca metal granules (shots). d. SEM photomicrograph of the $CaCO_3$ samples of experiment 5.

rich in HCl which could help to prevent the formation of the $Ca(OH)_2$ layer, and with an increase in Cl^- concentration from 0 (Exp-1) to 8 (Exp-2), then to 95 mM (Exp-4), the granules were dissolving in increasing amounts.

Experiment-5 was similar to experiment-4 but the MS solution (see Table 1) of Exp-5 also contained K^+ (5 mM), Mg^{2+} (1.5 mM), HCO_3^- (27 mM) and Cl^- (103 mM) ions at exactly the human blood plasma levels. Half a gram of starting Ca granules was completely dissolved and produced $CaCO_3$ precipitates (1.228 g) at a high process yield (98.15% of theoretical). The XRD data of the samples of experiments 4 and 5 (not shown) indicated $CaCO_3$ of relatively high crystallinity, individual XRD datum being indistinguishable from one another. However, the FTIR data of $CaCO_3$ produced in MS solution (Exp. 5) was showing the O–H stretching vibration at around 3700 cm^{-1}, as indicated in Fig. 1b. Based on observing the IR band at 1083 cm^{-1}, presence of very small amounts of vaterite may be suspected, although XRD data did not show this phase. The photomicrographs of the starting Ca granules and the calcite precipitated in Exp. 5 were given in Fig. 1c and d, respectively. The calcite crystals formed by adding 25 mM calcium granules into the MS solution (Exp. 5) had a mean particle size of around 5 μm, exhibiting a high degree of agglomeration, displayed nanosize steps and kinks reminding a diffusion-controlled crystal growth kinetics on their surfaces, and by this way, they differed from the clean and smooth-surfaced rhombic morphology of calcite synthesized in distilled water as reported by Matijevic et al. [38]. The first five experiments (of Table 2) also helped to explain why an aqueous solution with a Cl^- concentration close to 100 mM was needed for use with the Ca metal granules/shots. Human blood plasma contains 103 mM Cl^-, therefore, the findings of the first five experiments were also indicating us the way to develop a solution mimicking the ion concentrations of human blood plasma. The MS solutions of this study are not SBF (synthetic body fluid) solutions since they did not contain any Tris or Hepes, which are not present at all in the human metabolism.

Fig. 2 depicted the pH–time curves of the $CaCO_3$ synthesis experiments by using Ca granules. Ca metal granules were completely dissolved in experiments 4 and 5 at exactly the 11th minute. However, this specific time of dissolution would surely depend on the stirring speed (750 rpm) employed, as well as the volume and geometrical shape of glass bottles in which the reactions were performed throughout this study.

All of the above solutions and numbers may seem somewhat complicated at the first sight but they actually point to a very simple fact, which could most probably be explained by the below equations.

$$Ca(s) + H_2O(l) \rightarrow Ca^{2+}(aq) + 2OH^-(aq) + H_2(g) \quad (1)$$

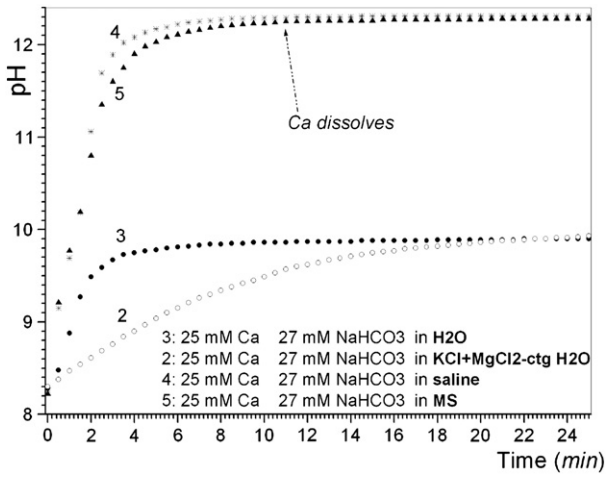

Fig. 2. pH–time curves for experiments 2, 3, 4, and 5 (the moment of dissolution of Ca granules were indicated for experiments 4 and 5).

$$Ca(s) + 2H_2O(l) \rightarrow Ca(OH)_2(s) + H_2(g) \quad (2a)$$

$$Ca^{2+}(aq) + HCO_3^-(aq) \rightarrow CaCO_3(s) + H^+(aq) \quad (2b)$$

$$Ca(OH)_2(s) + H^+(aq) + Cl^-(aq) \rightarrow Ca^{2+}(aq) + HCl + 2OH^-(aq) \quad (3)$$

Eq. (1) explains the evolution of H_2 gas and the observed rise in pH upon adding the calcium granules into the solutions. Eqs. (2a) and (2b) explain why the Ca granules did not dissolve in doubly-distilled water, and why the XRD data of Fig. 1 showed $Ca(OH)_2$. Calcium hydroxide, $Ca(OH)_2$, is extremely prone to conversion at its surface to calcite ($CaCO_3$), and even many "pure", commercial $Ca(OH)_2$ powders have measurable amounts of $CaCO_3$ in them, which can be readily confirmed by a simple FTIR run to be performed on those so-called pure and brand-new $Ca(OH)_2$ samples. Eq. (3) explains why the Ca-metal granules readily dissolved in blood plasma-like, mineralization solutions (MS), containing significant amounts (103 mM) of Cl^- ions, in such a short time by causing such a rapid rise in pH. There shall be a strong similarity between the behavior of magnesium metal [39–43] and calcium metal in this respect.

3.2. Synthesis of CaP in HCO_3^--free solutions by using Ca metal

Ca metal shots/granules were not expected to fully react in water only containing HPO_4^{2-} ions. In other words, in the absence of Cl^- ions, the granules would be easily covered with Ca-hydroxide and/or Ca-carbonate and would stop reacting. This expectation was tested in experiment 6 (Table 2). 25 mM of calcium granules stirred in water only having 10 mM Na_2HPO_4 did not dissolve completely, but the pH of the solution was able to rise above 12 and the small amount of precipitates formed were found, by XRD (Fig. 3a), to be comprised of biphasic mixtures of cryptocrystalline apatitic CaP (PCA) and calcite.

Experiments 7 and 8 were performed to study the effect of Ca/P molar ratio, i.e., 1.667 and 2.50, in reacting Ca granules with the MS solutions free of HCO_3^- ions. Both of these experiments produced cryptocrystalline apatitic calcium phosphate (PCA) samples in solutions with final pH values greater than 12 (Fig. 3a and b), without any calcite. It was important to notice the characteristic stretching vibration of the O–H group at 3571 cm^{-1} in the IR data (Fig. 3b) of the sample of Exp-8. Carbonates detected in the samples of Fig. 3a and b were due to the small amounts of dissolved HCO_3^- present in the distilled water (not previously boiled) used. Calcium granules reacted completely by the end of the 11th minute as shown in Fig. 3c.

The MS solutions of experiments 7 and 8 had 115 mM Na^+, 103 mM Cl, 5 mM K^+, 1.5 mM Mg^{2+} and 10 mM HPO_4^{2-}, and in both experiments one would be able to freely change the Ca content without disturbing the concentration of any other ion in the solution; i.e., another advantage of using Ca metal in CaP synthesis. This would not be possible if one were using, for instance, $CaCl_2 \cdot 2H_2O$ as the calcium source.

Experiments 7 and 8, therefore, showed a simple way of producing cryptocrystalline (some call it poorly-crystalline or poorly-crystallized or nanocrystalline) apatitic CaP powders at RT, in a very short 25 min, without employing any external pH control technique (such as drop-wise addition of a strong base such as NH_4OH, NaOH, KOH,or LiOH) at an in situ solution pH of 12. Exp-8 had the nominal, solution Ca/P molar ratio of 2.5, which was equal to that of blood plasma. Bacteria cannot grow at a solution pH of 12, but they definitely can if the synthesis solutions were at neutral pH (6.8 to 7.6). This is another advantage of using Ca metal in PCA synthesis.

Experiments 9 and 10 (Table 2) were replacing the Na_2HPO_4 used in experiments 7 and 8 with $(NH_4)_2HPO_4$, while keeping all the other synthesis parameters unchanged. Although the presence of NH_4 ions in a synthesis system claiming to mimic the ions and

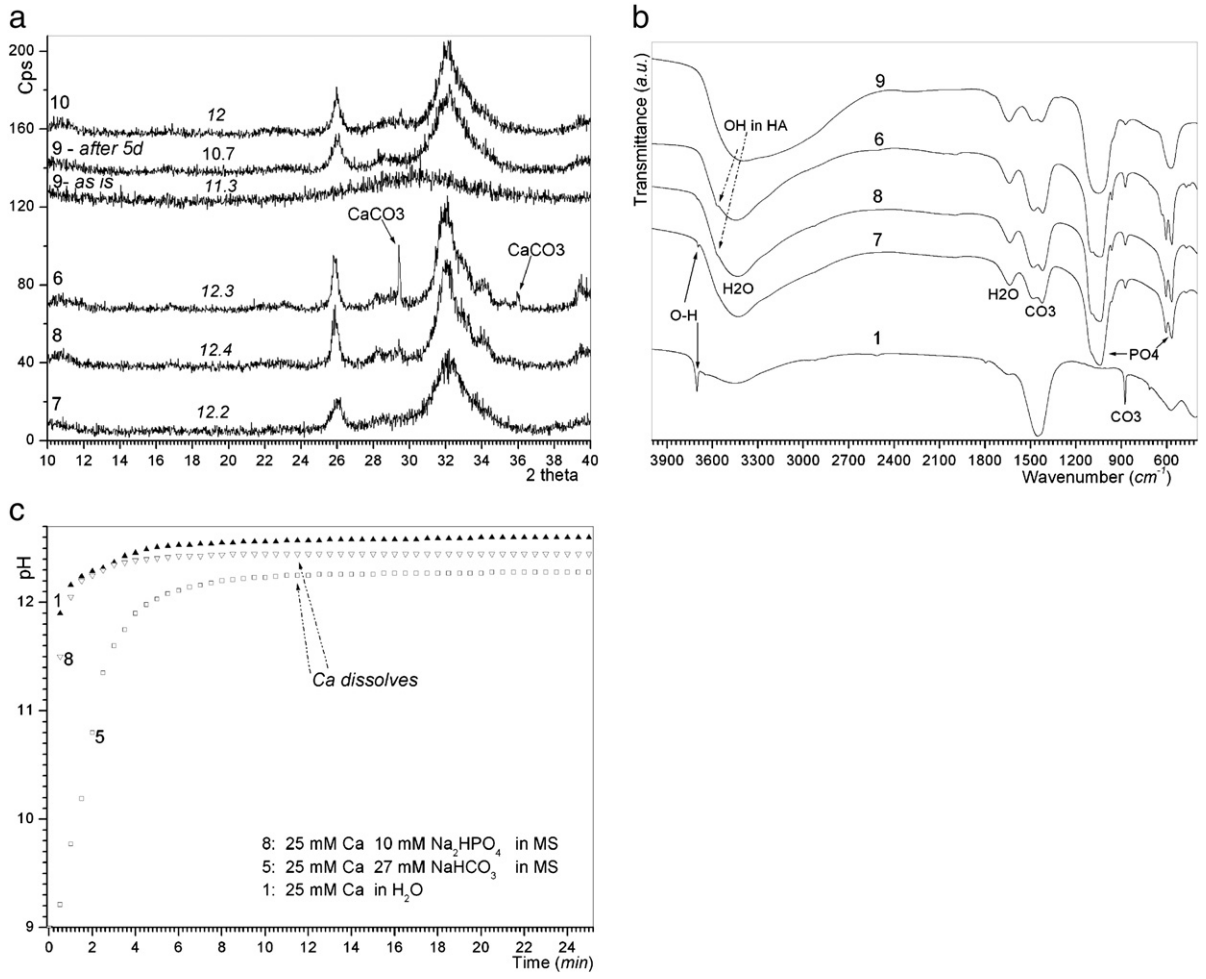

Fig. 3. a. XRD traces of the samples of experiments 6, 7, 8, 9, and 10 (solution pH values, at the end of 25 min of stirring, were shown on the traces). b. FTIR traces of the samples of experiments 1, 6, 7, 8, and 9. c. pH–time curves for experiments 1, 5, and 8 (the moment of dissolution of Ca granules was indicated by the arrows for experiments 5 and 8).

ion concentrations in blood plasma would not be acceptable, experiment 9 produced amorphous calcium phosphate (ACP) at the Ca/P molar ratio of 1.667 and the final pH value of 11.3.

It was quite easy to distinguish between the ACP and PCA phases by using their FTIR data, as exemplified by the IR traces of experiments 9 and 7 in Fig. 3b, respectively. In the IR data of ACP samples the phosphate bands over the range of 660 to 490 cm^{-1} do not show that splitting, which was otherwise observed in PCA samples. When the Ca/P molar ratio was increased to 2.5 in experiment 10, the produced powders were not ACP but PCA. The solution pH in this experiment was 12. Upon repeating the experiment 9, but aging the formed precipitates in the mother solution for 5 days at RT (solution pH dropping to 10.7 from 11.3, in 5 days), followed by filtering and drying, the obtained powders were consisted of PCA, not ACP, as shown in Fig. 3a. This was quite an expected result since ACP was not a stable phase (even in its mother liquor over a period of 5 days) and it acted as a precursor to PCA, as previously shown by Cazalbou et al. [44].

3.3. Synthesis of CaP in HCO_3^--free solutions by using $CaCl_2 \cdot 2H_2O$ instead of Ca metal

Upon replacing the Ca metal with $CaCl_2 \cdot 2H_2O$, the pH values of synthesis solutions drastically suffered from this change. Experiment 11 in comparison to experiment 6 showed that drastic drop in solution pH from 12.3 to 5.9. At such a low pH (5.9), it was inevitable to form DCPD (dicalcium phosphate dihydrate; brushite; $CaHPO_4 \cdot 2H_2O$). The comparison of the XRD and FTIR data of experiments 6 (with Ca metal in water) and 11 (with Ca-chloride in water) was given in Fig. 4.

Experiments 12 through 15 tested the formation of calcium phosphates in water and HCO_3^--free MS solutions by using Ca-chloride

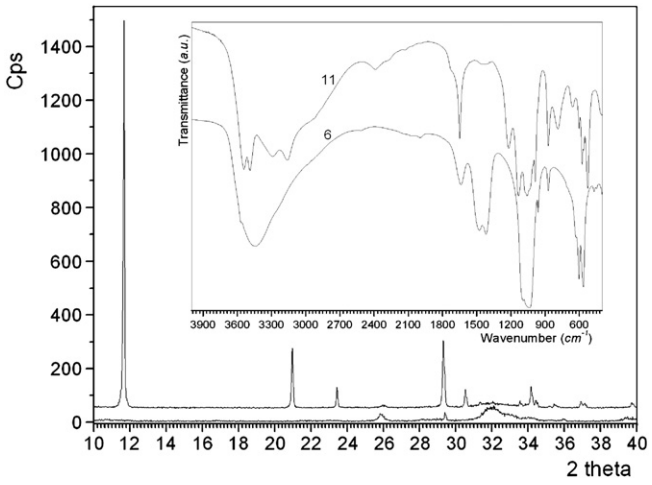

Fig. 4. Combined XRD and FTIR traces for the samples of experiments 6 and 11 (the bottom XRD trace for PCA of experiment 6, the XRD trace for DCPD of experiment 11 shown on top).

and diammonium hydrogen phosphate as the starting chemicals. In these experiments solution pH values remained between 5.7 and 6.5, and the obtained precipitates contained DCPD as the major phase.

If one used Ca-chloride dihydrate instead of Ca metal, as the calcium source, to synthesize CaP in HCO_3^--free plasma-like solutions, mildly acidic DCPD would be the major phase obtained. The readers shall compare the synthesis conditions and the results of experiments 8, 10 and 13 with one another.

3.4. Synthesis of ACP in MS solutions by using Ca metal

Experiments 16 through 18 tested the synthesis conditions closest to the ionic concentrations of the human blood plasma, by using the metallic Ca granules. In experiment 16; calcium, phosphate (HPO_4^{2-}), bicarbonate (HCO_3^-), potassium, chloride, and magnesium ion concentrations were made identical with that of blood plasma, but in that experiment the sodium concentration was equal to 124 mM. In experiment 18, on the other hand; bicarbonate (27 mM), sodium (142 mM), magnesium (1.5 mM), potassium (5 mM) and chloride (103 mM) ion concentrations were identical with that of blood plasma. In other words, experiments 16 through 18 tested the MS solutions given in Table 1 under three different choices. The combined XRD and FTIR data of the resultant ACP samples were given in Fig. 5a. The second inset of Fig. 5a confirmed the absence of the octacalcium phosphate (OCP, $Ca_8(HPO_4)_2(PO_4)_4 \cdot 5H_2O$) phase in the samples of experiments 16 to 18. At such high solution pH values it would be very difficult, if not impossible at all, to observe acidic OCP. The sample of experiment 18 showed the presence of a small amount of calcite ($CaCO_3$) phase in its XRD data. However, when we duplicated experiments 16 through 18, and left the precipitate-containing solutions overnight without stirring, followed by filtering and drying, the resultant XRD data of especially experiment 18 did not show that second phase of calcite. All three samples (16–18) depicted the characteristic XRD pattern of ACP. The Ca metal granules in experiments 16 through 18 all dissolved/disappeared at around the 11th minute. When experiment 18 is performed (i.e., experiment 19) in doubly-distilled water (containing 10 mM HPO_4^{2-}, 27 mM HCO_3^-, and 47 mM Na^+), instead of the MS solution, Ca metal granules did not dissolve and no precipitates were obtained. This again proved the role of Cl^- ions, as explained by Eqs. (1) through (3) above.

Fig. 5b showed the pH-time curves for experiments 16 through 19. The curves for experiments 16 through 18 in this figure, as well as the previous pH–time curves (Fig. 3c), exhibited a nonlinear increase of pH in a time dependent manner and they were approximated (TableCurve, v1.10, Jandel Scientific, 1993) by the logistic dose–response function ($y = a + [b/(1 + (x/c)^d)]$), for which the experimental parameters were given below in Table 3.

The SEM photomicrographs of samples obtained from experiments 16 and 18, were given in Fig. 5c and d, respectively. It should be noted that these are filtered and dried samples, they were not even lyophilized upon separation from their mother liquors. Regular drying causes agglomeration of individual particles or moieties.

Nevertheless, it was apparent from Fig. 5c and d that the average particle diameter in these X-ray amorphous, carbonated and

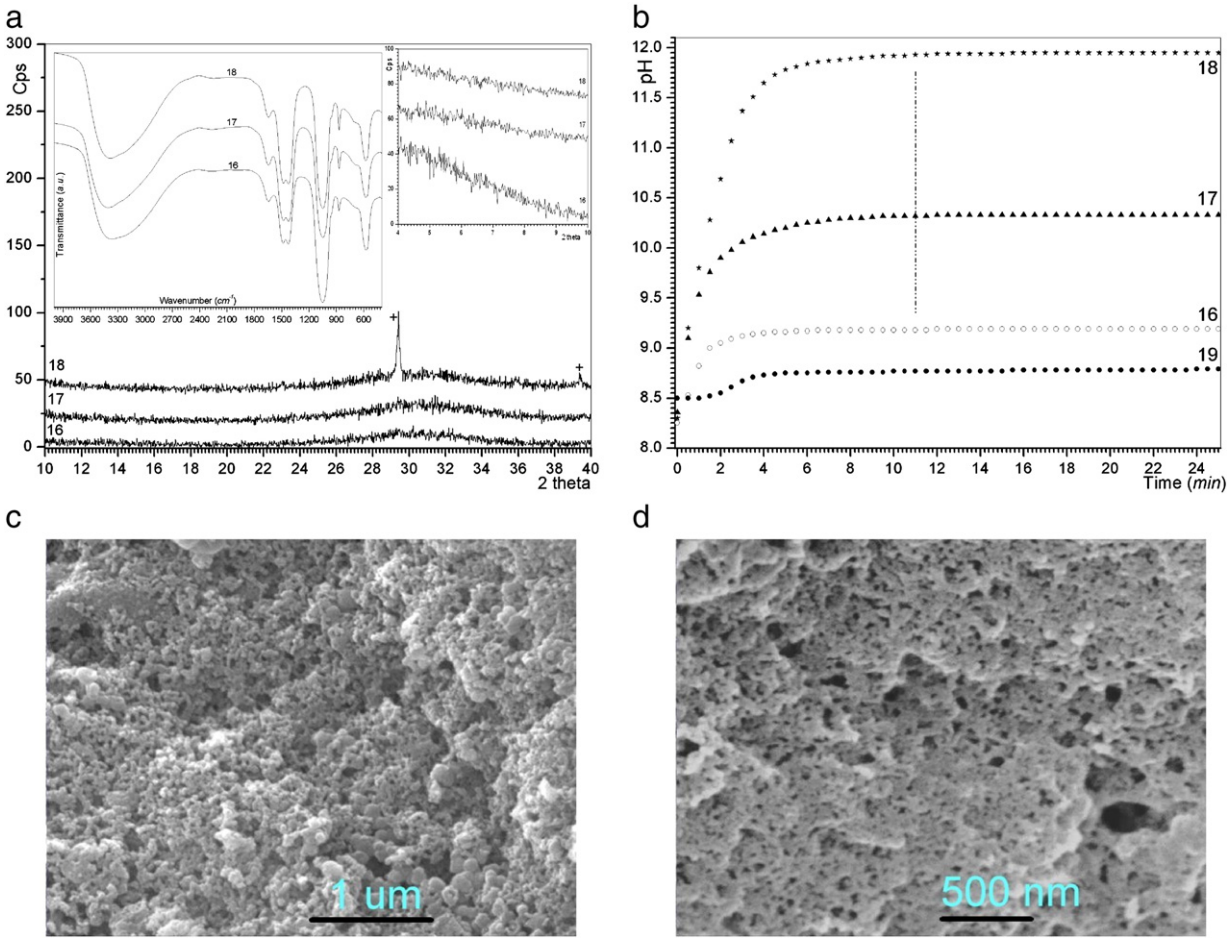

Fig. 5. a. Combined XRD and FTIR traces for the samples of experiments 16, 17, and 18 ($CaCO_3$ peaks were indicated by + in the XRD trace of experiment 18). b. pH–time curves for experiments 16, 17, 18, and 19 (the dissolution time of Ca granules was indicated by the straight dashed line). c. SEM photomicrograph of the sample of experiment 16. d. SEM photomicrograph of the sample of experiment 18.

Table 3
Results of logistic dose–response curve fitting on the pH–time curves.

Parameters	Exp 16	Exp 17	Exp 18	Exp 8	Exp 5
a	8.2495	8.3653	8.4338	12.6960	8.4163
b	0.9421	2.0039	3.5657	−3.7066	3.9064
c	0.7841	0.7827	1.3204	0.0222	1.4376
d	−1.9020	−1.3032	−1.7912	0.4083	−1.8735
r^2	0.9994	0.9985	0.9936	0.9794	0.9922
Fit Std Error	0.0043	0.0139	0.0608	0.0739	0.0760

mesoporous CaP powders was pretty much less than 70 nm. This is the particle size directly observed by the SEM, not the crystallite size. Crystallite sizes cannot be determined by using the Scherrer equation while using the XRD data of X-ray amorphous samples (Fig. 5a).

The concentration of Ca metal added into the MS solutions (starting from 2.5 mM in experiment 16 and going up to 25 mM in experiment 18) was found to be quite influential on the final pH values attained in syntheses. When the Ca concentration was kept equal to that of the blood plasma (i.e., 2.5 mM in exp. 16), the pH of the solution has risen only to 9.2 and stabilized at that value. By increasing it to 12.5 mM (i.e., 5 times that of plasma in exp. 17), the pH rose to 10.3, and the pH increased to 12 when the Ca concentration in the MS solution was increased to ten times that of the blood plasma (exp. 18).

The conditions of Exp-16 was of pivotal significance for this study, since the Ca^{2+}, HPO_4^{2-}, HCO_3^-, Mg^{2+}, K^+, Cl^- concentrations of this experiment were identical with those of human blood, and moreover, no foreign ions such as nitrate, ammonium and acetate were introduced to the synthesis process. As shown by the data of Fig. 5b, maintaining a literally constant pH in CaP synthesis, without employing any pH control (such as adding bases or acids to keep the pH constant), was never shown before to be possible. These define the novelty and practicality of the approach of using Ca metal as the sole calcium source in CaP synthesis.

3.5. Synthesis of ACP in MS solutions by using Ca metal, ammonium phosphates and ammonium carbonate

The influence of the use of $(NH_4)_2HPO_4$ and NH_4HCO_3 salts, instead of Na_2HPO_4 and $NaHCO_3$ was also tested in synthesizing CaP powders by using Ca metal granules. Such a direct comparison was necessary. Experiments 20 through 24 (of Table 2) all produced ACP powders in MS solutions. The use of Na-phosphate or Na-bicarbonate (as shown in experiments 20 and 21) kept the solution pH at above 10, but when both of Na_2HPO_4 and $NaHCO_3$ were replaced by $(NH_4)_2HPO_4$ and NH_4HCO_3 the solution pH values dropped to about 9.3 to 9.5 (experiments 22 through 24). Of course, the solutions used in these experiments could not mimic the physiological solutions, since they contained significant amounts of ammonium ions which are not found in blood plasma. The XRD and FTIR data of experiments 22 through 24 were shown in Fig. 6. However, the direct comparison of Exp-18 and Exp-24 showed that it would be possible to produce carbonated ACP powders, by using Ca metal, at pH values of 12 and 9.5, respectively, without using any external pH adjustment controls.

3.6. Synthesis of PCA in MS solutions at pH 7 without using Ca metal

Experiments 25 through 30 of Table 2 studied the synthesis of CaP in MS solutions, without using Ca metal. These experiments were planned to show what difference the use of Ca metal would really cause in comparison to the more commonly preferred calcium ion sources, such as $CaCl_2 \cdot 2H_2O$, calcium acetate monohydrate ($Ca(CH_3CO_2)_2 \cdot H_2O$), $Ca(NO_3)_2 \cdot 4H_2O$, and $Ca(OH)_2$. Fig. 7 showed the XRD traces of samples

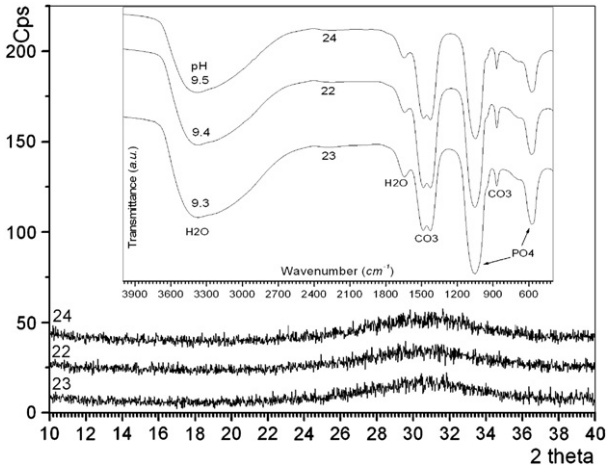

Fig. 6. Combined XRD and FTIR traces for the samples of experiments 22, 23, and 24.

obtained in experiments 25 through 29, all indicating PCA. The inset in Fig. 7, on the other hand, exhibited the IR traces of the samples of experiments 25 through 27. The IR traces of experiments 26, 28 and 29 were very similar to one another, and they all exhibited much less carbonate ion presence (according to the qualitative IR data) in comparison to, for instance, the sample of experiment 27.

MS solutions were working perfectly well, at the stated ion concentrations, in providing a reaction pH of exactly 7.0 for Ca-chloride, Ca-acetate, or Ca-nitrate; without a need for any external pH adjustments by acids or bases of any kind. Ca metal granules made it possible to synthesize ACP or PCA powders at pH values higher than 7.0, without needing any base additions for pH control, in MS solutions.

To synthesize PCA by using Ca metal granules, we found that one needed to eliminate HCO_3^- from the MS solutions. Using $CaCl_2 \cdot 2H_2O$ in doubly-distilled water or HCO_3^--free MS solutions containing phosphate ions, without any pH adjustments, would never allow the synthesis of PCA, since the pH of the solutions was lower than neutral (i.e., 7) and would thus only be suitable for the crystallization of brushite ($CaHPO_4 \cdot 2H_2O$) phase, as also shown in this study.

3.7. Ca metal granules or $Ca(OH)_2$ in MS solutions?

XRD and FTIR analysis of the sample obtained in experiment 30 (Table 2), which opted for 25 mM $Ca(OH)_2$ to be added into the typical MS solution of this study, tried to provide an answer to the question of this section. Fig. 8a compared the XRD traces of all the samples

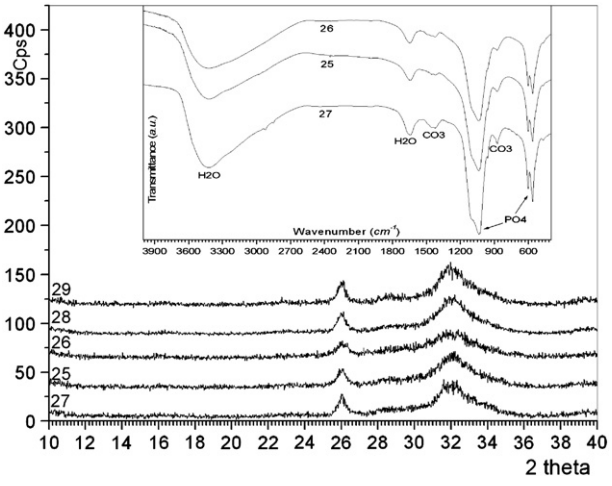

Fig. 7. Combined XRD and FTIR traces for the samples of experiments 25 through 29.

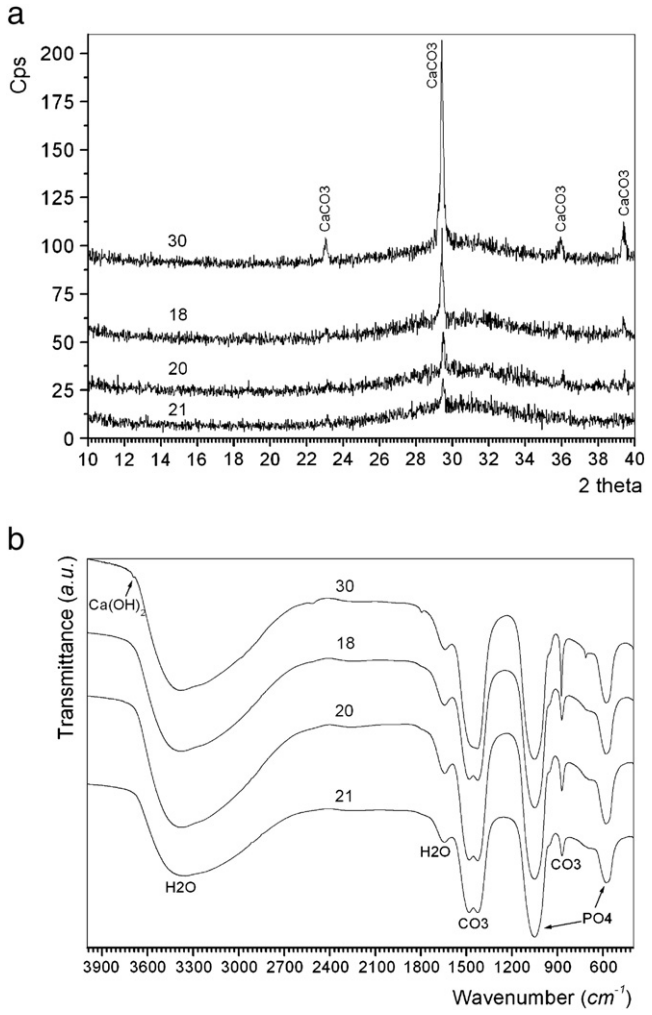

Fig. 8. a. XRD traces of the samples of experiments 18, 20, 21, and 30. b. FTIR traces of the samples of experiments 18, 20, 21, and 30.

of this study which comprised of a biphasic mixture of ACP and $CaCO_3$ after 25 min of stirring at RT in the MS solutions. The main comparison should actually be made between the sample 18 (25 mM Ca) and sample 30 (25 mM $Ca(OH)_2$) in the chart of Fig. 8a, since ammonium ions were present in the solutions of samples 20 and 21. Solution-wise, samples 20 and 21 do not compare well with those of samples 18 and 30. When Ca metal in experiment 18 was replaced by $Ca(OH)_2$ in experiment 30, while keeping all the other synthesis parameters constant, the amount of the secondary phase of $CaCO_3$ significantly increased (Fig. 8a). The FTIR data of the same experiments were given in Fig. 8b. Fig. 8b provided the evidence that the sample of experiment 30 was also poisoned with unreacted $Ca(OH)_2$, i.e., presence of the $Ca(OH)_2$-specific IR band recorded at around 3650 cm^{-1}. Moreover, the sample of experiment 30 showed the characteristic IR bands of the calcite phase at 2513, 1798, 875 and 712 cm^{-1}. In the duplicate experiments same results were obtained meaning that Ca-hydroxide was not able to completely react to form ACP in the MS solutions by fully consuming itself.

3.8. Significance of synthesizing CaP in mineralization solutions free of Tris or Hepes

Human blood, which provides the necessary nutrients to the trabecular/cancellous bones and the dentine of teeth, does not contain Tris (or Hepes), nitrate, acetate and/or ammonium ions. Therefore, it would be difficult to classify the synthesis (or coating) processes using Tris–HCl (or Hepes–NaOH) buffered solutions and especially the synthesis methods using one or more of the starting chemicals of Ca-nitrate tetrahydrate, Ca-acetate monohydrate, ammonium hydroxide, diammonium hydrogen phosphate or ammonium dihydrogen phosphate as properly mimicking the physiological processes [45–49].

Ammonium-, nitrate- and acetate-free synthesis recipes (especially those of experiments 7, 8, 16, 17 and 18) given in Table 2 of this study provided easy-to-reproduce and quite simple procedures to synthesize PCA (cryptocrystalline apatitic CaP) and ACP (X-ray amorphous CaP) powders at RT in glass media bottles, without requiring special reactor designs and pH adjustment/control measures. It would be naïve to assume that the PCA or ACP synthesized in such blood plasma-like solutions would be free of ionic substitutions of Na^+, K^+, Mg^{2+}, CO_3^{2-} and Cl^- ions at the crystallographic Ca, PO_4 and OH sites of hydroxyapatite structure. In a follow up study, we will publish the results of ICP-AES (inductively-coupled plasma atomic emission spectroscopy) analyses on such samples in comparison to PCA or ACP synthesized in synthesis media free of K^+, Mg^{2+} and Cl^- ions.

The ionic strength of the synthesis solutions (after the addition of Ca metal granules) of experiments 7, 8, 16, 17 and 18 of this study was adjusted to be 167.83, 184.5, 139.5, 171.5 and 211.5 mM, respectively. If one were to prepare an aqueous solution comprising 2.5 mM Ca^{2+}, 1 mM HPO_4^{2-}, 142 mM Na^+, 5 mM K^+, 1.5 mM Mg^{2+}, 27 mM HCO_3^- and 103 mM Cl^- (i.e., the exact ion concentrations of human blood plasma) then the ionic strength of that solution would have been 148.5 mM. The ionic strengths higher than 148.5 mM were intentionally chosen in this study to facilitate the synthesis of larger amounts of PCA or ACP powders.

The influence of synthesis pH on the CaP formation seemed to be not receiving the required attention in the previous literature. To the best of our knowledge, there are very few studies to mention the basicity of apatitic CaP forming in solutions with pH values around 11. The current study obtained pH values from 9 to 12 without adding any base. Liu et al. [50] used the Ca-nitrate/$(NH_4)_2HPO_4$ route and studied the ACP and apatitic CaP precipitation at pH 10 to 11, whereas the high pH values in that study were apparently obtained by NH_4OH additions. Liu et al. [50] study was not designed to measure the basicity of the CaP formed. The lack of previous studies on the basicity of apatitic CaP may even force the field researchers to think that apatite (which is basically a hydroxyl-containing phosphate in its formula and structure) is not a compound with a significantly basic surface, which is not true. However, the work of Tsuchida et al. [51] deliberately and quantitatively studied the surface basicity of apatitic CaP, by again using the Ca-nitrate/$(NH_4)_2HPO_4$ route of synthesis (with ammonia additions during synthesis) and found that (*i*) the solution pH had the greatest influence on the Ca/P ratio of apatitic CaP produced and (*ii*) the basic site density in apatite depended only on the Ca/P ratio of the sample. Therefore, the current study using Ca metal provided a very simple method of synthesizing CaP at the high pH values (from 10 to 12) studied separately by Liu et al. [50] and Tsuchida et al. [51].

Most of our samples produced at pH values 9 to 12 were of poor crystallinity. Nelson [52] investigated the reason for the poor crystallinity of Na^+- and CO_3^{2-}-containing apatitic CaP samples (usually prepared by high temperature ($90°<T<250$ °C) processes) by using TEM and found out that the reason was not a decrease in overall particle size but the fact that each particle consisted of agglomerates of small crystalline domains each having a different orientation. The domain sizes appeared to decrease with an increase in the carbonate content and could become as small as 8 nm. Nelson [52] also found out that the simultaneous incorporation of Na^+ and CO_3^{2-} ions into the apatitic CaPs resulted in an increased rate of dissolution for the solid. The formula for the Na- and CO_3-doped CaP could be as complex as $Ca_{10-x}Na_x[(PO_4)_{6-x}(CO_3)_{4x/3}][(OH)_{2-2x/3}]$, where $0 \le x \le 3$ [53]. A similar formula for K- and CO_3-doped CaP shall be expected. Nonstoichiometric CaP phases would usually have slightly higher

aqueous solubility with respect to their perfectly stoichiometric counterparts [54].

Although the size of the Mg^{2+} ion (0.066 nm) is quite smaller than that of Ca^{2+} (0.101 nm), magnesium ions can substitute for Ca in a number of CaP phases, including whitlockite ($Ca_3(PO_4)_2$) [55]. The incorporation of Mg into amorphous CaP has been relatively well studied. Termine et al. [56] found that the elapsed time between the precipitation of ACP and its solution-mediated transformation into cryptocrystalline apatitic CaP (PCA) may be increased considerably with the addition of small amounts of Mg^{2+} ions. The current study was not focused on the hydrothermal transformation of ACP into PCA or vice versa, however our synthesis solutions (MS) contained Mg^{2+}.

The author's lab has been the first to synthesize cryptocrystalline apatitic CaP powders in Tris-buffered SBF (synthetic body fluid) solutions (by using Ca-nitrate) at 37 °C and to show (via ICP analyses) that Mg and Na were indeed incorporated into the obtained powders [46]. Such biomimetic apatite powders were also shown to possess unprecedented high stability against thermal decomposition [46].

For readers who may ask the question of why one would need a solution pH as high as 9.2 (as in Exp-16) to synthesize CaP mimicking the physiological processes, it is a well-known fact that alkaline phosphatase (ALP) enzyme is secreted in bones by the osteoblast cells while depositing nanosize apatitic CaP crystals, and the optimum pH of ALP secretion is between 9.5 and 10.5 [57–59]. Synthesis procedures described for Experiments 16 and 17 in Table 2 were both able to produce the ACP phase at or around this biomimetic pH value of ALP secretion.

It was also shown in this study, in contrast to some previous reports, that the use of synthetic polymers, which do not have any place in the human metabolism, was not necessary at all to synthesize ACP in aqueous media [60].

Magnesium metal was already tested [39–43] as a starting material for biomedical scaffolds, the current study may initiate the use of Ca metal for the same purpose.

4. Conclusions

Metallic calcium was used for the first time in synthesizing $CaCO_3$, poorly-crystalline (cryptocrystalline) apatite (PCA) or X-ray amorphous calcium phosphate (ACP) powders.

Calcium phosphate synthesis with metallic Ca was tested both in doubly-distilled water and in water containing ions found in human blood.

The use of metallic Ca eliminated the need for external pH control in calcium phosphate synthesis solutions in the form of adding strong bases such as NaOH, KOH, LiOH or NH_4OH.

The use of metallic Ca made it possible to synthesize PCA or ACP powders in solutions completely free of foreign ions such as ammonium, nitrate or acetate, which are not encountered in human blood.

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