Chemical transformation of some biologically relevant calcium phosphates in aqueous media during a steam sterilization

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The purpose of this study was to investigate the effect of steam sterilization on some biologically relevant calcium phosphates: $CaHPO_4 \cdot 2H_2O$ (DCPD), calcium deficient apatite (CDA) and biphasic calcium phosphate (BCP). Suspensions of 0.2 g of each calcium phosphate compound with 5.0 ml of deionized water were prepared and steam sterilized in an autoclave (20 min at 121 °C). After sterilization the suspensions were filtered and the dried solids characterized with scanning electron microscopy, IR-spectroscopy and X-ray diffraction. The pH and calcium concentrations of the filtrates were determined with ion selective electrodes. Similar measurements were made with the same samples which were not sterilized. The sterilization procedure was found to result in the dehydration of DCPD and hydration of calcium oxide incorporated into the BCP. Solution pH was observed to change from 7.3 to 5.5 for the solutions in equilibrium with DCPD and from 8.5 to 10.6 for those in equilibrium with BCP. Minor changes both with the solid and liquid phases were found to occur during the steam sterilization of CDA. These results indicate that steam sterilization may have different effects on different calcium phosphate suspensions: it can result in dehydration of DCPD, fast hydration for CaO in BCP, but no significant effect on CDA.

1. Introduction

Various calcium phosphates are widely used in surgery because they have an excellent biocompatibility both with hard and soft tissues of the human body [1, 2]. Prior to the clinical use in dentistry and surgery, all the materials must be sterilized. Different cleaning and sterilization procedures, e.g. gamma and laser irradiation, plasma cleaning, steam sterilization, chemical treatment with ethylene oxide and some detergents, are commonly used for this purpose [3–7].

In spite of a wide application of calcium phosphates in dentistry and medicine, surprisingly there are only a few publications devoted to the influence of the sterilization conditions on the chemical properties of calcium phosphates. For example, the surface of pure stoichiometric hydroxyapatite (HA) was found to remain unchanged after steam sterilization in an autoclave, but sterilization with gamma irradiation resulted in driving off the weakly bonded surface water and distortion of surface phosphate complexes of HA [8]. A steam sterilization of dental gypsum at 132 °C and 121 °C was found to result in its partial dehydration [9].

Three biologically relevant calcium phosphates: $CaHPO_4 \cdot 2H_2O$ (DCPD), calcium deficient apatite (CDA) and biphasic calcium phosphate (BCP-containing 60% HA and 40% β-$Ca_3(PO_4)_2$) were chosen for the sterilization experiments. These materials were studied because BCP is widely used in surgery as an artificial bone substitute [2, 10–12] and it is produced from CDA, while the CDA is often prepared from DCPD by chemical interaction of the latter with NaOH [10–12]. So, it appears to be both interesting and important to study the influence of steam sterilization to the chemical properties of the above calcium phosphates.

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2. Materials and methods

2.1. Calcium phosphates

BCP, consisting of a mixture of HA and β-$Ca_3(PO_4)_2$, was developed earlier for use as a bone substitute [10–12]. It was obtained from CDA, while the latter was obtained from DCPD by its hydrolysis in alkaline media according to the well-known procedure [13–15]. In order to produce BCP, the CDA obtained during hydrolysis should be sintered for at least one h at temperatures over 1000 °C.

All the calcium phosphates investigated were characterized with the X-ray diffraction technique. BCP was found to consist of 60% HA and 40% β-$Ca_3(PO_4)_2$ [11, 12], while DCPD (purchased from Merck) and CDA were found to contain no admixes of other phases.

2.2. Steam sterilization procedure and measurements

Water suspensions of the above calcium phosphates (0.2 g of the solid and 5 ml of deionized water) were sealed in small (10 ml) bottles and steam sterilized in an autoclave according to the standard procedure used in dentistry (20 min at 121 °C) [9]. Briefly, a single run of the steam sterilization procedure in an autoclave includes three stages: temperature increasing up to 121 °C during 15 min with simultaneous pressure increasing from 995 to 2150 mbar, sterilization at 121 °C during 20 min at pressure of 2150 mbar, drying at 90 °C in vacuum (pressure of 120–155 mbar) during 15 min followed by fast (1–2 min) temperature reduction to 25 °C. Thus, the total time of a single run of the steam sterilization procedure is about 60 min.

Solution pH of the suspensions was measured both before and after the steam sterilization followed by additional measurements during the next two weeks. After this period the suspensions were filtered through a 1.0 μm filter (Millipore). Calcium concentrations in the liquid phases were determined using a calcium selective electrode (Model 97–20 ionplus, Orion Research Inc.). Chemical and structural compositions of the solids were studied with Fourier transform infrared spectroscopy (FTIR) (Magna-IR 550, Nicolet) in the range of 400–4000 cm^{-1} (3–5 mg of the solid were mixed with 300 mg of KBr followed by pellets preparation), X-ray diffraction (XRD) (Diffract 5000, Siemens) within 2θ value of 10–60 degrees (CuK_α radiation was used) and scanning electron microscopy (SEM) (JSM 6300, JEOL) in the secondary electron mode (acceleration voltage 15 kV) as described before [11, 12]. Similar measurements were made both for the suspensions before sterilization and for the suspensions without sterilization.

2.3. Thermo-gravimetric analysis

In order to obtain information about the chemical changes which occur during preparation of BCP from CDA, a standard thermo-gravimetric analysis (TGA) was performed, as described in the literature [16–18]. Briefly, 15–25 mg of CDA were placed into a small platinum crucible and heated from room temperature to 1050 °C with a heating rate of 5.0 °C/min. A TGS 2 system 4 (Perkin Elmer) installation was used for this purpose. Numeric values of mass decreasing were permanently followed and recorded during the heating.

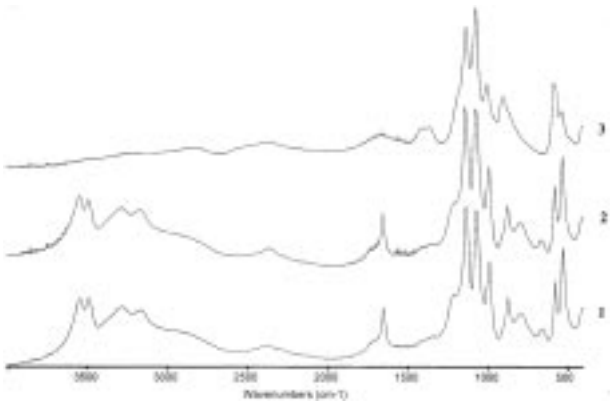

Figure 1 FTIR spectra of DCPD: 1, initial DCPD; 2, non-sterilized DCPD; 3, sterilized DCPD (monetite).

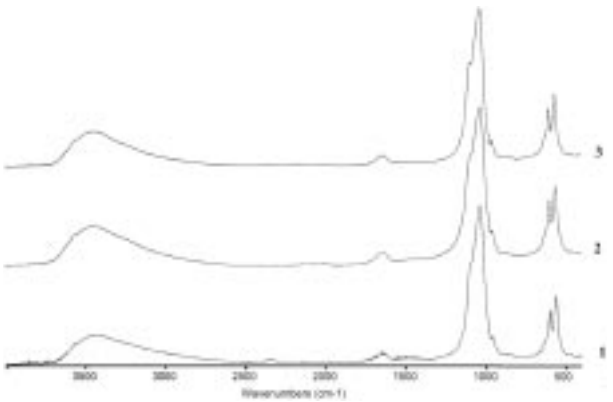

Figure 2 FTIR spectra of CDA: 1, initial CDA; 2, non-sterilized CDA; 3, sterilized CDA. Small peaks of carbonate may be seen in the regions of 1400 and 870 cm^{-1}.

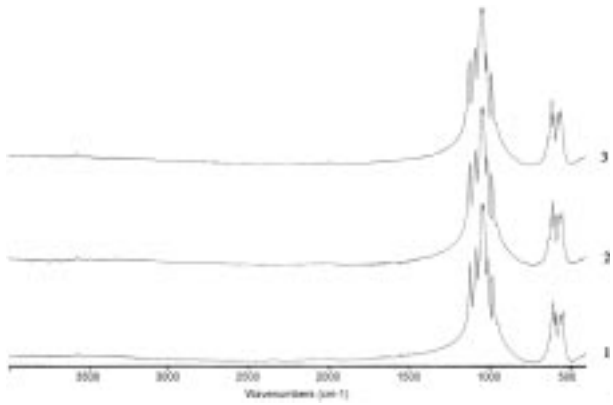

Figure 3 FTIR spectra of BCP: 1, initial BCP; 2, non-sterilized BCP; 3, sterilized BCP.

3. Results

3.1. FTIR investigations

The results obtained with the FTIR investigations on samples of DCPD, CDA and BCP before and after suspension in water and after the steam sterilization are shown in Figs 1–3, respectively. No differences before and after suspension in water (curves 1 and 2 on Figs 1–3) were seen for all the calcium phosphates investigated. For the steam sterilized samples, no differences were observed for CDA and BCP (curves 1–3 on Figs 2 and 3), while some changes were observed with DCPD (curves 2 and 3 on Fig. 1). Spectrum 3 on Fig. 1 was identified as belonging to monetite ($CaHPO_4$-anhydrous form of DCPD) [2]. The results demonstrate that no significant chemical changes (within the sensitivity of FTIR technique) happen when DCPD, CDA and BCP were equilibrated with water for two weeks at room temperature. A single run of the steam sterilization procedure (20 min at 121 °C) also made no visible changes in the spectra of CDA and BCP, but resulted in complete dehydration of DCPD.

3.2. X-ray diffraction

XRD analysis for the calcium phosphates are shown in Figs 4–6, respectively. Like the results of the FTIR measurements, no differences before and after equilibration with water were observed for all the calcium phosphates investigated (curves 1 and 2 on Figs 4–6). For the steam sterilized samples (curves 2 and 3) some changes occurred only with DCPD (Fig. 4). Spectrum 3 on Fig. 4 appeared to be equal to that of monetite [2, 19]. Thus, the experimental results using XRD appear to be in perfect agreement with those using FTIR.

3.3. Scanning microscopy investigations

Investigations by SEM allowed the study of crystal sizes and shape for the above calcium phosphates. Typical pictures obtained are shown in Figs 7–15. According to the results obtained, there are no differences in crystal sizes and shape before and after equilibration in water for DCPD (Figs 7 and 8 respectively), while a different type of crystal is typical for the sterilized sample of DCPD (Fig. 9). Equilibration in water for two weeks resulted in a decrease in crystal size of CDA (Figs 10 and 11), while the steam sterilization procedure did not cause any changes (Figs 10 and 12).

Equilibration of BCP with water appeared to result in some changes in BCP crystals. A comparison of BCP before (Fig. 13) and after (Fig. 14) water equilibration and steam sterilization (Fig. 15) showed some differences: the crystals resembled packs of needles among the former and ''broken'' crystals among the latter (Figs 14 and 15 respectively). Thus, except for that of the non-sterilized DCPD (Fig. 8), an interaction of the above calcium phosphates in water was found to result in some dissolution/precipitation (or re-crystallization) phenomena: they were minor for CDA and great both for BCP and the sterilized DCPD.

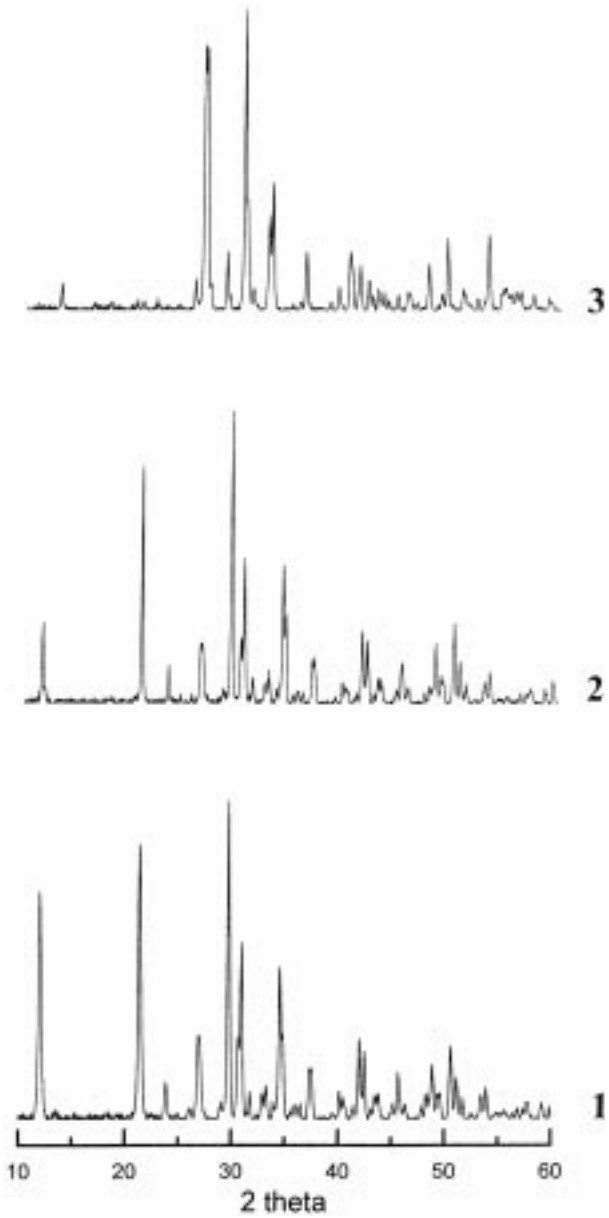

Figure 4 XRD spectra of DCPD: 1, initial DCPD; 2, non-sterilized DCPD; 3, sterilized DCPD (monetite).

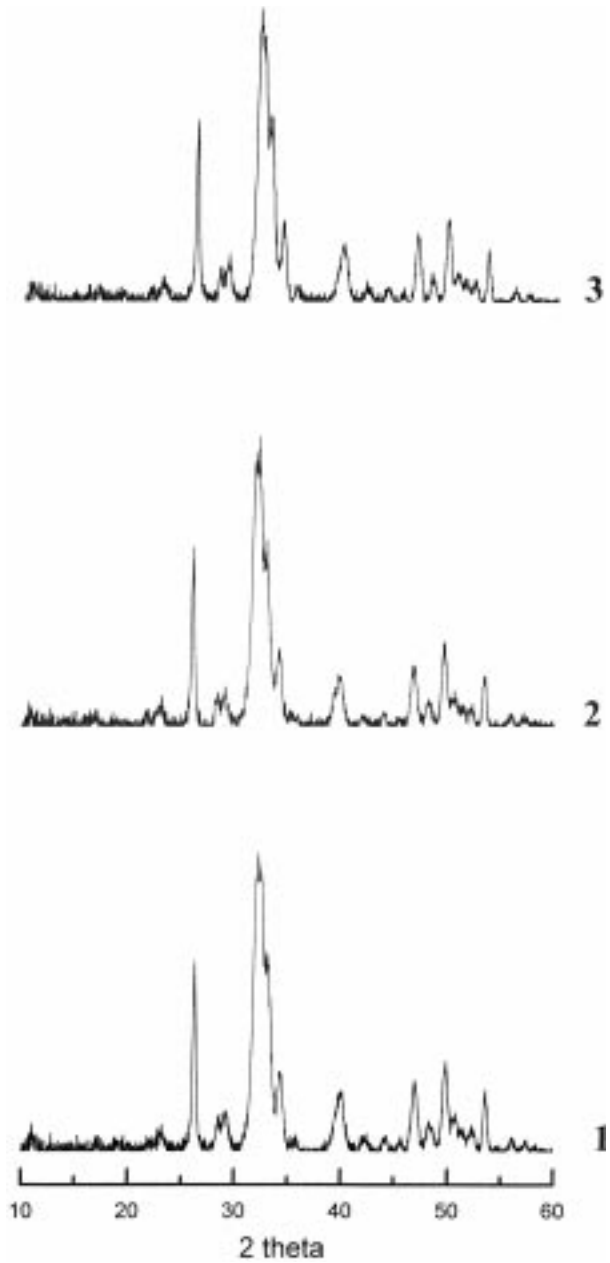

Figure 5 XRD spectra of CDA: 1, initial CDA; 2, non-sterilized CDA; 3, sterilized CDA.

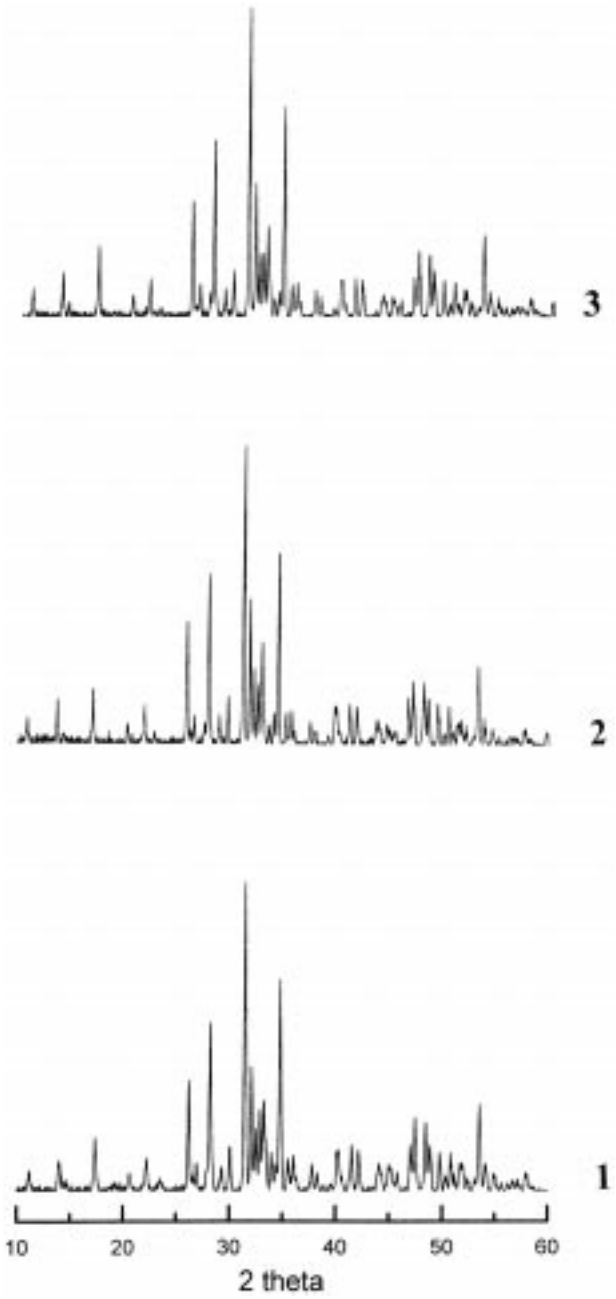

Figure 6 XRD spectra of BCP: 1, initial BCP; 2, non-sterilized BCP; 3, sterilized BCP.

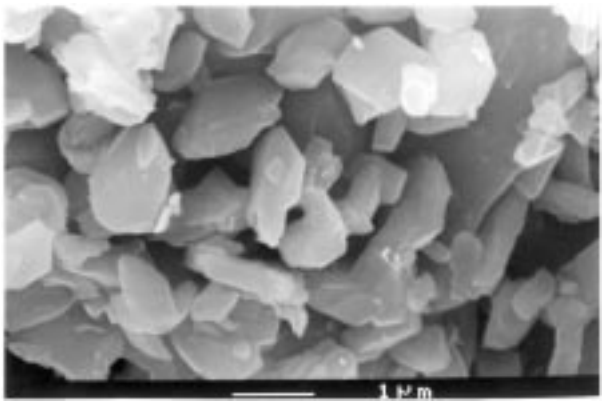

Figure 7 Crystals of the initial DCPD. Magnification × 15 000. Bar is 1 μm.

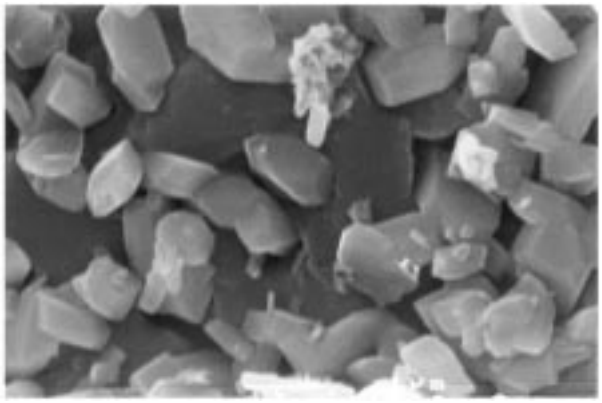

Figure 8 Crystals of the non-sterilized DCPD. Magnification × 15 000. Bar is 1 μm.

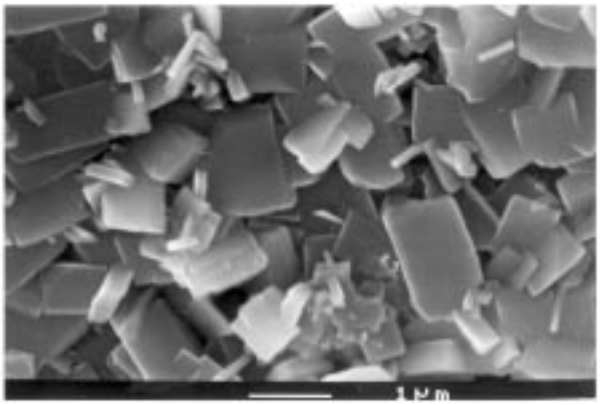

Figure 9 Crystals of the sterilized DCPD (monetite). Magnification × 15 000. Bar is 1 μm.

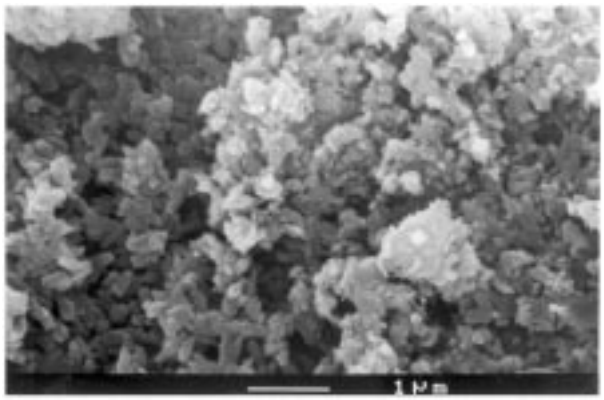

Figure 10 Crystals of the initial CDA. Magnification × 15 000. Bar is 1 μm.

3.4. Calcium analysis and pH

The results on calcium concentrations and pH values obtained for the solutions equilibrated with the calcium phosphates investigated are summarized in Table I. In order to explain the observed pH increase of the solutions equilibrated with BCP (Table I), additional sterilization experiments were performed. A suspension of BCP in water prepared as described in the experimental section was subjected to ten standard sterilization procedures (20 min at 121 °C in an autoclave). After each sterilization run, the BCP suspension was filtered and the pH of the liquid phase was measured. The solid phase was again suspended in fresh water followed by the next sterilization procedure. Ten successive sterilization runs

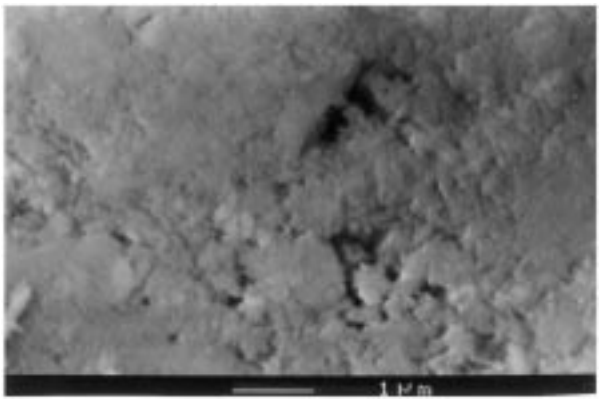

Figure 11 Crystals of the non-sterilized CDA. Magnification × 15 000. Bar is 1 μm.

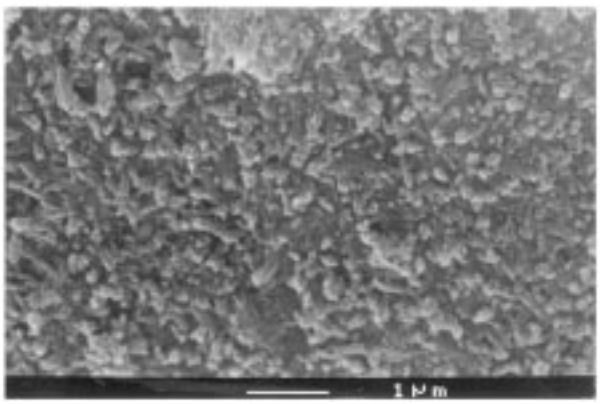

Figure 12 Crystals of the sterilized CDA. Magnification × 15 000. Bar is 1 μm.

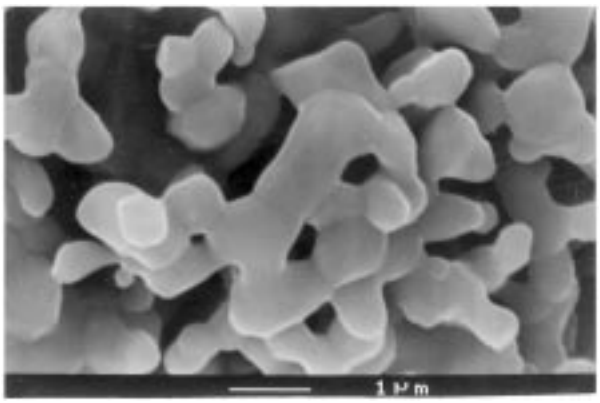

Figure 13 Crystals of the initial BCP. Magnification × 15 000. Bar is 1 μm.

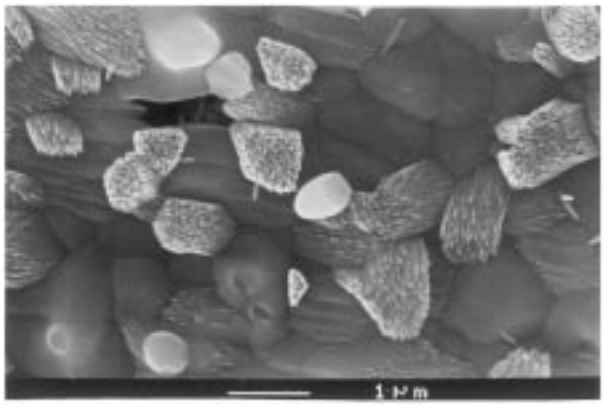

Figure 14 Crystals of the non-sterilized BCP. Magnification × 15 000. Bar is 1 μm.

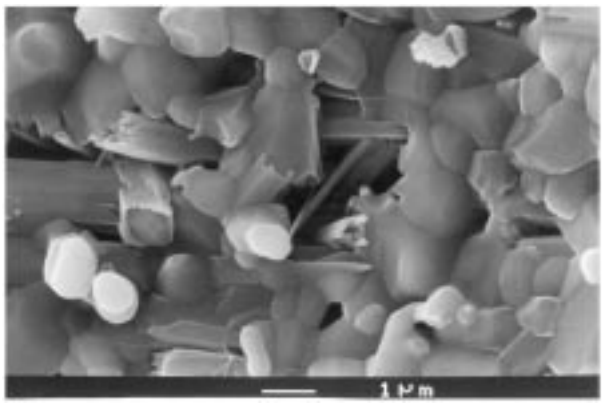

Figure 15 Crystals of the sterilized BCP. Magnification × 15 000. Bar is 1 μm.

were performed with the same sample of BCP (each time in fresh water). The results of pH measurements are summarized in Table II. The solid BCP, which had been subjected to ten sterilization procedures, was studied with FTIR, XRD and SEM. No changes were found if compared with the BCP samples subjected to only one steam-sterilization procedure (see Figs 3, 6 and 15).

4. Discussion

Three points should be emphasized in the results summarized in Table I. First, pH of solutions in equilibrium with all the calcium phosphates studied changed immediately after the sterilization and, except for BCP, remained more or less constant within next two weeks. Second, there is an essential difference between BCP and other calcium phosphates studied (DCPD and

TABLE I pH and calcium concentrations of the solutions equilibrated with calcium phosphates investigated

	Solution pH initial (29.09)	Solution pH 30.09.97	Solution pH 01.10.97	Solution pH 02.10.97	Solution pH 03.10.97	Solution pH 04.10.97	Solution pH 10.10.97	Solution pH 13.10.97	Ca^{2+} mol/l 13.10.97
DCPD	7.29	7.22	7.20	7.20	7.19	7.17	7.11	7.14	$4 \cdot 10^{-4}$
$DCPD_s$	7.29*	5.52	5.56	5.53	5.59	5.61	5.58	5.61	$5 \cdot 10^{-3}$
CDA	8.78	8.64	8.61	8.57	8.60	8.59	8.58	8.61	$4 \cdot 10^{-5}$
CDA_s	8.78*	8.20	8.18	8.13	8.09	8.06	8.02	8.01	$5 \cdot 10^{-5}$
BCP	8.51	9.80	10.18	10.46	10.59	10.63	10.57	10.56	$<10^{-5}$
BCP_s	8.51*	10.60	10.59	10.58	10.56	10.53	10.51	10.46	$<10^{-5}$

*pH measurements were made before the steam sterilization. Index $_s$ means the sterilized samples. All columns except the last one are devoted to solution pH followed during two weeks while the last column is devoted to the results on calcium concentration in the solutions measured at the end of the experiments only.

TABLE II Solution pH values for the ten-times sterilized suspension of one and the same BCP (each time in fresh water)

Number of water changing	Initial water	1	2	3	4	5	6	7	8	9
Solution pH after sterilization	10.49	7.36	6.17	6.37	5.87	6.31	6.19	6.10	6.12	6.15

CDA): solution pH for the sterilized (BCP_s) and non-sterilized (BCP) samples became equal after four days after the sterilization procedure (03.10) and slowly decreased during the next 10 days, while for both DCPD and CDA solution pH for the sterilized and non-sterilized samples remained different. The third point is valid for BCP only: as soon as a maximum in the solution pH was reached (immediately after the sterilization for the sterilized ($pH = 10.60$) and after four days for the non-sterilized BCP ($pH = 10.63$)), solution pH slowly began to decrease in a similar way (Table I). The last two points mean that the steam-sterilization results in changes of the BCP hydrolysis kinetics only (fast increase in pH during a single run of the sterilization procedure at 121 °C and slow increase in pH at room temperature). This was not observed for water suspensions of DCPD and CDA. So, some irreversible changes have obviously happened during their sterilization.

In the case of DCPD, experimental results on the FTIR, XRD and SEM investigations pointed to formation of anhydrous $CaHPO_4$ as a result of the steam-sterilization (Figs 1, 4 and 7–9, respectively). Thus, dehydration of DCPD was found to occur during the sterilization:

$$CaHPO_4 \cdot 2H_2O \rightarrow CaHPO_4 + 2H_2O \quad (1)$$

Both $CaHPO_4 \cdot 2H_2O$ and $CaHPO_4$ are known to be in equilibrium with water solutions, but at different temperatures: the former at $t < 50\,^{\circ}C$, while the latter at $t > 60\,^{\circ}C$ [2]. That is why dehydration of DCPD during the steam-sterilization appears to be in good agreement with the references [2]. On the other hand, according to the solubility phase diagram for the system $Ca(OH)_2$-H_3PO_4-H_2O at 25 °C, solubility of the anhydrous form is always a little bit less than that for DCPD within pH range of 4–8 [20]. The latter should always result in DCPD dehydration in water media at room temperatures. A small shift in solution pH from 7.29 to 7.11–7.14 over two weeks (see Table I) can not be seriously considered as a confirmation of the DCPD dehydration at room temperature (it may be attributed to a drift of an electrode potential), but it goes in the direction of the pH decrease, as happens after the sterilization.

According to reaction 1 no pH changes should occur during DCPD dehydration, while in reality, solution pH decreased from 7.2 to 5.6. The latter is responsible for calcium concentration increasing in the solution (the last column in Table I). Indeed, according to the solubility diagram, solubility of any calcium phosphate increases with pH decrease [20]. But why has the solution pH changed? According to the calculations made by Lemaître, solubility differences between DCPD and its anhydrous form may cause the pH drop for 0.2 pH units only [21]. On the other hand, Lemaître has calculated that if during the steam-sterilization only 0.064% of the initial DCPD transforms into HA and phosphoric acid (a hydrolysis of DCPD):

$$5CaHPO_4 \cdot 2H_2O = Ca_5(PO_4)_3OH + 2H_3PO_4 + 9H_2O \quad (2)$$

solution pH would be as low as 5.4 [21]. The latter value is very close to the experimentally found values of the solution pH within 5.5–5.6 (Table I). This assumption is also in good agreement with the solubility diagram of calcium phosphates: HA is known to be less soluble if compared with both DCPD and its anhydrous form under solution $pH > 4.5$ [20]. Such small values of HA obtained (0.064% in the solid phase) can be detected neither by FTIR nor by XRD. If the above explanation seems to be reasonable, one may expect that either the crystals of anhydrous $CaHPO_4$ obtained during the steam-sterilization of DCPD are covered (probably, in part only) with a very thin layer of HA (something like an epitaxial growth of HA on monetite) or the crystals of HA are formed by a heterogeneous nucleation somewhere in the bulk solution. In any case, the amount of HA obtained appears to be too small to be observed with SEM.

A difference between pH of solutions in equilibrium with CDA (pH within 8.0–8.6) and BCP ($pH \approx 10.6$) (see Table I) was observed. This may be attributed to some chemical changes that could occur within the CDA during sintering at 1050 °C. The results of the SEM investigations demonstrate significant changes in crystal sizes and shape during sintering (Figs 10 and 13). However, both FTIR and XRD techniques did not reveal the chemical changes.

In order to elucidate this question, the TGA analysis was performed. According to the results obtained, transformation of CDA into BCP results in total mass decreasing by 8.7%. Most of the mass decrease (8.15%) happens at temperatures below 350 °C and the rest of it (0.55%) happens at 650–700 °C. The former is due to elimination of traces of water and the latter due to carbonate [16–18]. Moreover, the carbonate ions substituted for phosphate in apatite structure (called type B carbonate) begin to decompose at 600 °C; the decomposition of carbonate ions substituted for hydroxide ions (type A carbonate) occurs at higher temperatures [17].

In spite of the carbonate evidence in CDA (it was not identified by XRD), its presence might be suspected as small peaks in the ranges of 1400 and 870 cm^{-1} in the FTIR spectra (Fig. 2). So, in agreement with Rey *et al.* [17], the thermal decomposition of the CDA investigated may be described like this:

$$Ca_{10-x}(HPO_4, CO_3)_x(PO_4)_{6-x}(OH)_{2-x} \rightarrow Ca_{10}(PO_4)_6(OH)_2 + Ca_3(PO_4)_2 + CaO + CO_2 + H_2O \quad (3)$$

In this case the differences in solution pH between CDA and BCP may be explained by the presence of 0.7% CaO in BCP. The latter easily transforms into calcium

hydroxide in water solutions and causes the solution pH increase.

The above hypothesis is supported by the results on ten-times sterilization (each time in fresh water) of one and the same sample of BCP (Table II). In the case of water changing, pH of the solutions equilibrated with BCP after subsequent sterilization procedures was found to decrease rapidly from an initial value of 10.49 to 7.36 and 6.17 after the first and the second water changing, respectively. After the third and following water changing, pH remained more or less constant (Table II). Small variations in pH values (within 5.9–6.3) obtained for the solutions after the third water changing seem to be random.

Based on the results obtained one may assume that practically all the CaO was transformed into $Ca(OH)_2$ and dissolved in water during the first sterilization. The latter resulted in solution pH increasing up to 10.49 (the second column in Table II). When the initial water solution was replaced with fresh water for the first time, only traces of CaO and/or $Ca(OH)_2$ were left in the wet solid phase of BCP. These traces of CaO and/or $Ca(OH)_2$ are likely to be responsible for a small pH increase to 7.36 found after the first water changing (the third column in Table II). As soon as all the CaO was removed after the second water changing, further water changing and sterilization procedures resulted in more or less constant solution pH (other columns in Table II).

Thus, the differences in solution pH found between CDA and BCP (Table I) are due to carbonate ions present in CDA. During BCP preparation traces of carbonates decompose into CaO and CO_2 according to reaction 3 and the presence of CaO in BCP results in solution pH increasing, while other solid components (HA and β-$Ca_3(PO_4)_2$) probably remain unchanged in aqueous media. This conclusion is in the good agreement with the references, because no chemical changes were found to happen during the steam sterilization of water suspensions of pure HA [8].

A permanent but slow pH decrease of the solutions equilibrated with BCP (Table I) is consistent with the results published in literature [22–24]. For example, a continuous pH decrease of solutions equilibrated with solid BCP and β-$Ca_3(PO_4)_2$ was found, while pH of solutions equilibrated with pure HA was stable. Moreover, equilibration of BCP in water solutions was found to result in an increase of HA/β-$Ca_3(PO_4)_2$ ratio in the solid phase [24]. The latter was attributed to dissolution of more soluble β-$Ca_3(PO_4)_2$ and precipitation of less soluble HA (a hydrolysis of β-$Ca_3(PO_4)_2$) according to an approximate reaction:

$$10Ca_3(PO_4)_2 + 6H_2O = 3Ca_{10}(PO_4)_6(OH)_2 + 2H_3PO_4 \quad (4)$$

No doubt, a similar process also occurs in our case and results in a pH decrease in the final stages of BCP equilibration in water. On the other hand, dissolution of CaO in water and its chemical transformation into calcium hydroxide result in solution pH increase. It would be reasonable to assume that when BCP comes in contact with water, dissolution of CaO happens much faster if compared with β-$Ca_3(PO_4)_2$ hydrolysis according to reaction 4. This results in a pH increase. As soon as all the CaO has been dissolved (a maximum in the solution pH has been reached), the above mentioned hydrolysis of β-$Ca_3(PO_4)_2$ appears to be the only chemical reaction possible and results in pH decrease. That is why pH of solutions equilibrated with BCP initially increase and later slowly decrease.

Finally, an influence of the steam sterilization to the properties of CDA should be discussed. As mentioned above, no changes both in structure and chemical composition of the solid phase after the sterilization were found both with FTIR and XRD (Figs 2 and 5 respectively) and some changes in crystal sizes were found with SEM (Figs 10–12). Equilibration of the non-sterilized sample of CDA in water during two weeks at room temperature was found to result in crystal sizes decreasing (Figs 10 and 11). This may be attributed to some re-crystallization (or dissolution/precipitation) processes happening within CDA in aqueous media. Such hypothesis has already been discussed in literature. For example, this idea was raised during a discussion of the experimental results on human dental enamel equilibration in slightly acidic (pH within 4.7–6.2) solutions of HCl [25]. According to the authors, poor crystallized non-stoichiometric carbonatapatite of human dental enamel may be dissolved (it is more soluble), while pure HA may be crystallized instead. This may cause some change in the solution pH [25].

On the other hand, a small drop in solution pH (from 8.6 to 8.2–8.0) is another difference between the sterilized and non-sterilized samples of CDA (Table I). This pH drop may be explained either by presence of traces of DCPD and their hydrolysis according to chemical reaction 2 during the steam-sterilization or by partial re-crystallization of CDA into less soluble HA [25] according to an approximate chemical reaction:

$$10Ca_9HPO_4(PO_4)_5OH + 8H_2O = 9Ca_{10}(PO_4)_6(OH)_2 + 6H_3PO_4 \quad (5)$$

Both ways result in a pH decrease. A slow but permanent pH decrease from 8.2 to 8.0 during the next two weeks in the sterilized sample of CDA (Table I) as well as the results of SEM investigations (Figs 10–12) seem to be in favor of reaction 5.

5. Conclusion

Thus, an influence of a steam-sterilization procedure in an autoclave (20 min at 121 °C) to the chemical transformations of three biologically relevant calcium phosphates in water media was investigated. The sterilization procedure was found to result in dehydration of DCPD and fast hydration of calcium oxide incorporated into the crystal structure of BCP with simultaneous changes in solution pH from 7.3 to 5.5 for the solutions in equilibrium with DCPD, and from 8.5 to 10.6 for those in equilibrium with BCP. Minor changes both in the solid phase and in the solution were found to occur during sterilization of CDA. So, steam-sterilization may have a different influence on calcium phosphates suspensions: it can result in dehydration (DCPD), fast hydration (CaO in BCP) or have no great influence (CDA).

Acknowledgments

We acknowledge Prof. Racquel Z. LeGeros (New York University) for her kind participation. We are grateful to Dr J. Lemaître (Ecole Polytechnique Fédérale de Lausanne, Switzerland) and Dr M. Bohner (Dr h.c. Robert Mathys Stiftung, Postfach, Switzerland) for the calculations and valuable discussions. The technical assistance of Mr S. Grolleau (Institut des Matériaux de Nantes) for the TGA analysis and of Mr P. Pilet (Nantes University) for the SEM investigations are gratefully acknowledged. This work was supported by Nantes University and Region Pays de Loire (post doctoral scholarship for S. Dorozhkin).

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Received 25 August 1998
and accepted 29 April 1999