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Amorphous calcium phosphates: Synthesis, properties and uses in biomaterials

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A B S T R A C T

This review paper on amorphous calcium phosphates (ACPs) provides an update on several aspects of these compounds which have led to many studies and some controversy since the 1970s, particularly because of the lack of irrefutable proof of the occurrence of an ACP phase in mineralised tissues of vertebrates. The various synthesis routes of ACPs with different compositions are reported and the techniques used to characterise this phase are reviewed. We focus on the various physico-chemical properties of ACPs, especially the reactivity in aqueous media, which have been exploited to prepare bioactive bone substitutes, particularly in the form of coatings and cements for orthopaedic applications and composites for dental applications.

Keywords:
Amorphous calcium phosphates
Synthesis
Characterisation
Biomaterials
Biomineralisations

1. Introduction

The term amorphous calcium phosphate (ACP) has different meanings. In its first usage ACP refered to amorphous calcium orthophosphate phases, and the core of this report will essentially deal with these substances. ACP is also used to refer to micelles of calcium phosphate (CaP) in milk and cheese, poorly defined domains in apatite ceramics and apatite nanocrystals of biological or synthetic origin and, sometimes, amorphous calcium polyphosphates. The environment of CaP phases in milk has some analogy with 'pure' ACP phases, despite strong interactions with casein, and will be briefly discussed in this report. Amorphous calcium polyphosphates are very different substances with concatenated phosphate groups, and these will not be discussed. Flaws in ceramics made from calcium orthophosphates often correspond to crystal defects and grain boundaries, and again we will not review these aspects. We include a short review and discussion of amorphous domains in nanocrystals, however, such 'amorphous' environments are rather different in nature from amorphous phases and a clear distinction has to be established between amorphous domains associated, for example, with surface structures or grain boundaries and amorphous phases. The ACP phases covered in this review are characterised by the absence of any periodic distribution of atoms.

ACP phases are one of the most frequent forms of CaP minerals in biological organisms [1]. They have been found, for example, in the mitochondria of eukaryote and prokaryote cells and ACP has been and is still considered a precursor phase of bone mineral in vertebrates, although this is highly controversial and is yet to be clearly established.

The ACP phase is an intermediate phase in the preparation of several CaPs by precipitation. ACP is present in many biomaterials and preparations. It is involved, for example, in coatings of metallic endoprostheses obtained by different techniques, either as a transitory phase or in the end product. It is used in self-setting injectable cements, where it is responsible for the setting reaction. In addition, ACP is found in several composite materials used in odontology as a remineralising phase for enamel and dentine and its inclusion in toothpaste formulations as a remineralising agent for early carious lesions has been proposed.

This short review on amorphous calcium phosphates will first report on some fundamental aspects related to ACP formation, composition, structure and physico-chemical properties. The main physico-chemical techniques used to characterise ACPs will then be presented. Finally, the different routes of synthesis of ACP and its main uses in the form of cements, ceramics, composites, coatings and colloidal suspensions for biomedical applications will be reviewed. Several other reviews have been published that present different conceptions and aspects of amorphous calcium phosphates [1–5].

2. Occurrence of ACPs

ACPs occur in many biological systems, especially in primitive organisms, where they are believed to serve as a reservoir of calcium and phosphate ions. Among the various biogenic amorphous minerals those mainly constituted of calcium and phosphate are

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most abundant in teeth and the exoskeletal structures of marine invertebrates [6].

Growing interest in ACPs arose in the 1970s, due to the possible involvement of these compounds in the bone of vertebrates. The question of its occurrence during bone mineral development, before final crystallisation of the hydroxyapatite (HA)-like phase, has led to some discussion and controversy, although it remains undetectable even at the earliest stage of tissue formation [2,4,5,7–12]. Until now, the existence of ACPs in vertebrates has not been demonstrated experimentally except in specific locations, such as the inner ear structures of embryonic sharks and mammalian milk [2,6]. Based on X-ray radial distribution function analysis carried out on various bone samples, including those of embryos, Grynpas et al. concluded, however, that the chemical and structural changes observed could not be accounted for by the presence of ACP [9]. Recent works on bone and teeth minerals have suggested the presence of a transient amorphous mineral precursor and a universal strategy for calcium carbonate-based and CaP-based biomineralisation in both vertebrates and invertebrates [10,11,13–15]. Using several techniques, such as Fourier transformed infrared (FTIR) specroscopy, Raman and X-ray absorption near edge structure (XANES) micro-spectroscopy, high resolution scanning electron microscopy (SEM) and transmission electron microscopy (TEM), several authors have recently presented some evidence of transient ACP in several mineralised tissues such, as newly formed (outer) murine tooth enamel, newly formed fin bones of zebrafish and in the early intramembranous mineralisation of murine calvaria tissue [13–15]. However, because of a lack of irrefutable proof, the question of the occurrence of an ACP phase in such newly mineralised tissues of vertebrates remains unanswered.

In addition, characterising the first formed mineral deposits (transient amorphous mineral phases) in a tissue without altering them during sample preparation and/or analysis (for example by dehydration, irradiation or the use of solvents) is challenging. Such protocols can lead to the dehydration of nanocrystals and loss of their hydrated surface structure, which might be transformed into amorphous-like domains. In contrast, other analyses on wet and/or poorly preserved samples can lead to the conversion of amorphous phases into more stable apatite or octacalcium phosphate (OCP) phases.

Complementing these works related to biogenic ACP as a transient mineral phase, we can also find in the literature several in vitro studies on the precipitation of CaPs from electrolyte solution, contributing to our understanding of the formation of CaP biominerals [16–19]. The authors set-up in vitro conditions allowing ACP formation as a transient phase; ions and/or proteins involved in biomineralisation can be introduced into such in vitro model solutions or systems.

3. Different ACPs

The literature provides many references to different ACPs, essentially distinguishable by their Ca/P atomic ratio.

Amorphous tricalcium phosphate (ATCP), with an atomic Ca/P ratio of 1.5 and the chemical formula (I) $Ca_3(PO_4)_2{\cdot}nH_2O$, is most widely found in amorphous precipitates obtained in alkaline media (pH range 9–11). This composition corresponds to a well-defined compound and also seems to be found in ACP formed at high temperature. In fact, in the absence of mineral ions other than Ca^{2+} and PO_4^{3-} the composition of ACP is restrained to chemical formula (I) for charge balance reasons.

In more acidic solutions ACPs can contain HPO_4^{2-} ions instead of PO_4^{3-}, leading to a lower Ca/P ratio. Ratios as low as 1.15 have been obtained, however, such phases are unstable and convert very rapidly into dicalcium phosphate dihydrate (DCPD) ($CaHPO_4{\cdot}2H_2O$). A second 'stable' ACP, termed ACP2, has been proposed by Christoffersen et al. to explain the initial variation in pH of a solution during the transformation of ACP into crystalline CaP phases, such as OCP and calcium-deficient apatite [16]. Based on TEM analysis, ACP2 was identified as a separate amorphous phase with a floccular morphology and no electron diffraction pattern, compared with the spherular morphology of the first amorphous phase (ACP1). In experiments at 30 and 42 °C the Ca/P ratio of the early formed solid phases in solutions varied between 1.35 and 1.38.

In non-aqueous or ethanol–water media ACP phases with a much lower Ca/P ratio than composition (I) can be obtained, corresponding, for example, to amorphous OCP [$Ca_8H_2(PO_4)_4{\cdot}nH_2O$] or amorphous dicalcium phosphate (DCP) ($CaHPO_4$) [20–22]. The preparation of these ACP phases will be detailed in the synthesis of ACPs section of this paper.

ACP phases with a Ca/P ratio higher than 1.5 can only be obtained in the presence of foreign ions, most frequently carbonate and oxide ions. This is the case, for example, of ACP phases formed during plasma spraying of HA, which can contain oxide ions. Many other foreign ions may be incorporated into ACPs, and in these cases consideration of the Ca/P ratio to describe the ACP phase has no meaning.

However, the composition of even the most frequent form of ACP, i.e. ATCP, can change on ageing. According to Heughebaert, internal hydrolysis can occur in ATCP gels, leading to the formation of $HPO_4^{2-}$and OH^- ions [23,24]:

$$H_2O + PO_4^{3-} \rightarrow HPO_4^{2-} + OH^- \quad (1)$$

This leads to a range of compositions represented as:

$$Ca_9(PO_4)_{6-x}(HPO_4)_x(OH)_x$$

This reaction would precede the crystallisation of calcium-deficient apatite, considered to occur when $x = 1$. Evidence for the existence of $HPO_4^{2-}$and OH^- ions in ATCP has been recently provided using solid-state nuclear magnetic resonance (NMR) [25].

The present review paper reports studies mainly related to the most frequent form of ACP, i.e. ATCP.

4. Structure of ACP

The structure of ATCP was first determined by Betts and Posner based on the radial distribution function [26,27]. A short range order was evidenct in ATCP, corresponding to $Ca_9(PO_4)_6$ units with an average diameter of 0.95 nm, often referred as 'Posner's clusters'. These clusters correspond to a local arrangement of calcium and phosphate ions existing in the structure of apatites. It was later suggested that these clusters in fact possessed a S_6 symmetry. A representation of the Posner's cluster is given in Fig. 1a [28]. This arrangement is analogous to that existing in several other crystalline phosphates, such as apatites, OCP and β-tricalcium phosphate (TCP); the relationship between the original Posner's cluster and apatite structure is represented in Fig. 1b. Theoretical investigations of the stability of different calcium and phosphate clusters with an increasing number of ions have confirmed that Posner's clusters are the most stable arrangement [29]. Analogous arrangements have been found in intracellular biological calcified tissues and in high temperature ACP obtained from plasma spraying HA, although it has been suggested that larger clusters could exist [30,31]. The formation of Posner's clusters has been established for ATCP and supposes that there is no HPO_4^{2-} in the ACP. The effect of HPO_4^{2-} ions on the structure of Posner's clusters is unknown, as is the effect of biologically relevant foreign ions like carbonate, magnesium and pyrophosphate. Recent ab initio calcu-

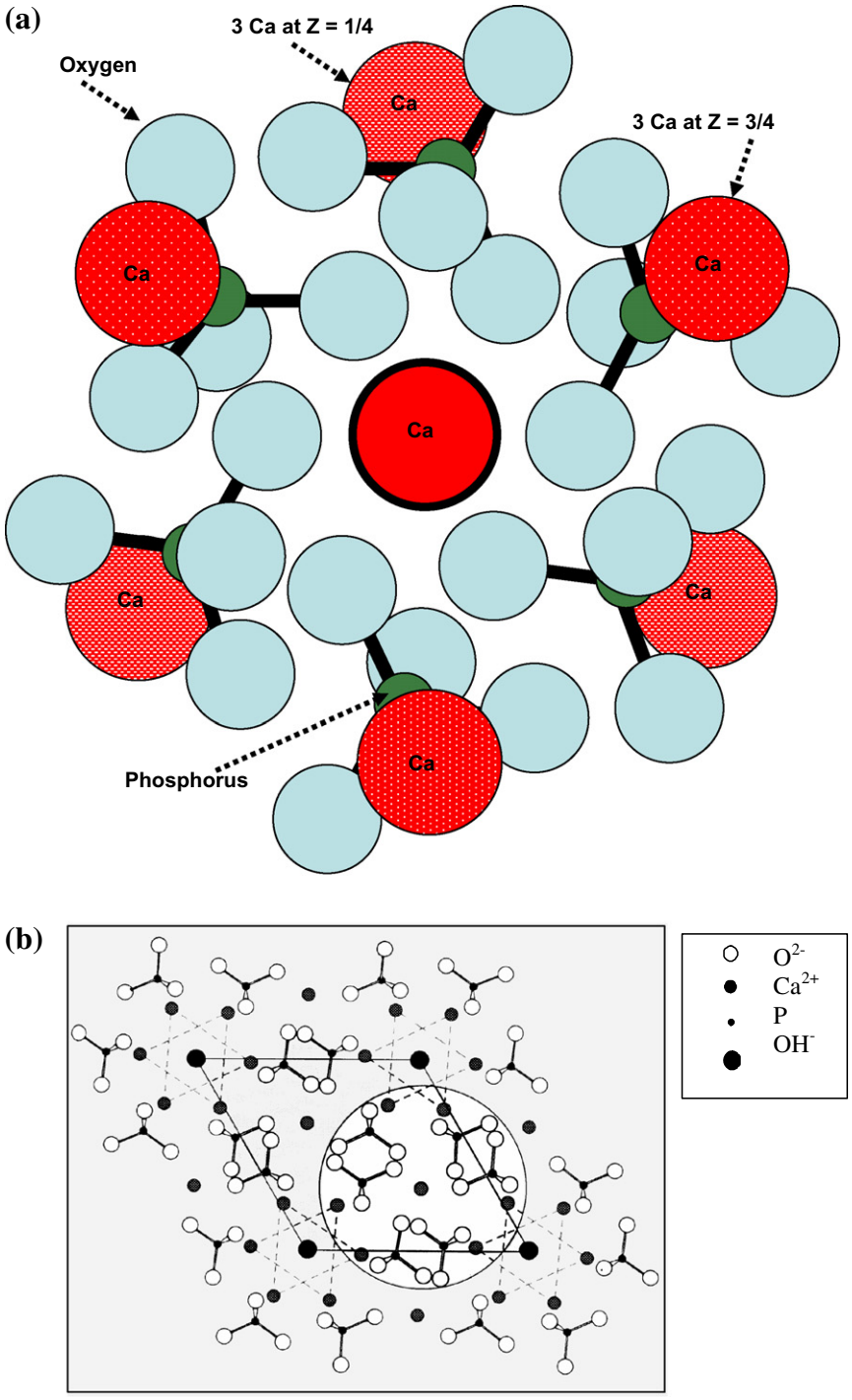

Fig. 1. Structure of the amorphous calcium phosphate. (a) Representation of Posner's cluster with a S_6 symmetry according to Treboux et al. [28]. Three calcium ions are superimposed, forming a column on the C_3 axis of the figure located at $z = 0$, 1/2 and 1. Two other groups of three calcium ions are at the periphery of the cluster at $z = 3/4$ and $z = 1/4$ positions. Phosphorus atoms of phosphate groups are at $z = 1/2$ position, with two of the oxygen atoms of the phosphate tetrahedrons. The global chemical composition corresponds to $Ca_9(PO_4)_6$. (b) Original model of Posner's cluster showing its relationship with the apatite structure.

lations suggest that Posner's clusters with six protons and six OH^- ions are stable, although such structures with a $Ca_9(HPO_4)_6(OH)_6$ composition have never been experimentally found [32].

Other investigations of the environments of ions in ATCP have been made by solid-state NMR and FTIR or Raman spectroscopy and will be discussed in the appropriate section.

The environment of calcium ions in ACP has also been investigated by extended X-ray absorption fine structure (EXAFS) spectroscopy and the data obtained seem in agreement with the model proposed by Posner [33].

The arrangements of clusters into larger structures are not well known. In fact, precipitated ATCP contains a large amount of water, considered to be present in part in the inter-cluster space, allowing the association of Posner's clusters into larger spherical units of 20–300 nm diameter [3]. A second type of water, more loosely bound, would be simply adsorbed onto these units. It has been

shown that spherical associations were preceded by disk-shaped associations corresponding to gel-like flocculates progressively transforming over time into more stable spherical units [34]. The morphology of ACP is still used as a distinctive feature to detect this phase in various systems, although it appears insufficient.

Determination of the specific surface area of ATCP generally leads to surprisingly low numbers. This is related to the larger spherical associations of Posner's clusters and the apparent hindrance to nitrogen adsorption of these inner surfaces [35,36].

5. Physico-chemical properties of ACP

5.1. Solubility

Determination of the solubility of ACP is a delicate problem and several different solubility products have been proposed, essentially concerning ATCP (Table 1). Some of these solubility products are lower than that of crystalline α-TCP and seem erroneous as the free-energy of formation of a crystalline substance is generally lower than that of an amorphous one with the same composition, which thus should lead to a higher solubility of the amorphous phase, which is generally found. One reason for these discrepancies is the instability of ACP and its propensity to crystallise into apatite or other phases, although Meyer and Eanes have shown that the ion activity product of ACP in aqueous solution remained constant over a long period and for a large range of pH values [2,37]. A second difficulty is related to the initial composition of ACP, especially its HPO_4^{2-} ion content or that of other ionic impurities such as Mg^{2+} and CO_3^{2-} ions, which are not usually reported. A last identified difficulty is the change in composition of ACP on ageing and the development of internal hydrolysis without any apparent structural change.

The best experimental approach to determining the solubility product of ATCP uses recent preparations with a large solid/solution ratio to reach a rapid solubility equilibrium before hydrolysis occurs.

5.2. Thermal stability

Unlike solubility, the thermal stability of ATCP is well documented. On heating ATCP first loses water. Two types of water loss occur, corresponding to loosely bound water molecules adsorbed on the surface of ATCP agglomerates and more strongly bound inter-cluster water molecules, respectively. The first loss is essentially reversible, whereas the second is mostly irreversible [46,47].

Table 1
Solubility products of ATCPs compared with those of α-TCP, β-TCP and two ACPs with Ca/P = 1.35.

Type of ACP (conditions)	Solubility product [$-\log(K_{sp})$] [calculated for $Ca_3(PO_4)_2$]	References and observations
ATCP (20 °C)	25.2	[37]
ACP 1[a] (Ca/P = 1.35)	25.5	[38] (~32% of total P as HPO_4^{2-})
ACP 2[a] (Ca/P = 1.35)	28.3	[38] (~32% of total P as HPO_4^{2-})
ATCP (25 °C, pH 7–9)	25.5	[39] (from the abstract, with 4% of total P as HPO_4^{2-})
ATCP (18 ± 3 °C)	26.5	[40]
ATCP	24.8	[41]
ATCP (25 °C)	25.7	[42]
ATCP (25 °C)	23.9	[43] (with 0.52 wt.% CO_3^{2-})
β-TCP	28.9	[44]
α-TCP	25.5	[45]

[a] These ACP differed in their ageing times.

Lyophilised precipitated ATCP crystallises into α-TCP at 630 °C. The initial formation of a metastable high temperature form when crystallisation of an amorphous compound occurs has been reported in many other systems. This phenomenon is known as 'Ostwald's step rule' or the law of successive reactions. In any crystallisation process the generally observed state is not the most stable state but the least stable state that is closest in terms of free energy change to the original state [48]. High temperature ACP crystallises generally at a higher temperature (around 700 °C) leading, according to Feng et al., to a mixture of HA, tetracalcium phosphate (TTCP) and CaO [49]. Ranz observed that recrystallisation of high temperature ACP leads to HA in the presence of water vapour and oxyapatite in vacuum [31]. In any case, α-TCP has been reported as a transitory phase, unlike low temperature ATCP.

Differential thermogravimetric analysis (DTA) and differential scanning calorimetry (DSC) studies show a narrow exothermic crystallisation peak, which can be used to estimate the amount of ACP in mixtures with apatite or other compounds. This crystallisation point is sensitive to the presence of impurities: it moves towards higher temperatures in the presence of carbonate, magnesium or pyrophosphate ions (the three major ionic stabilisers of ACP). This displacement is associated with a broadening of the exothermic peaks, which might possibly become difficult to observe [23].

5.3. Evolution in aqueous media

ACP is converted into hydroxylated apatite in aqueous media. This reaction has been studied in suspensions and gel-like states, on just precipitated wet samples and on lyophilised and heated powders [2,23,24,50,51]. Some common features can be distinguished: an induction period is generally observed during which the amorphous state is preserved; crystallisation then occurs rather rapidly and follows a sigmoid evolution [51]; the conversion of ATCP to apatite is 'autocatalytic', i.e. in the presence of apatite seeds the induction period is no longer observed and conversion is more rapid; in most conversion reactions occurring at physiological pH the apatite obtained is hydroxylated, calcium-deficient and contains hydrogen phosphate species. Several differences can be observed depending on the initial state of the ACP.

5.3.1. Conversion in the gel-like state

Heughebaert et al. and Guégan distinguished two events in the hydrolysis of ATCP gels: a microcalorimetric analysis curve for ACP conversion to apatite at 25 °C is presented in Fig. 2 [23,24,51]. The

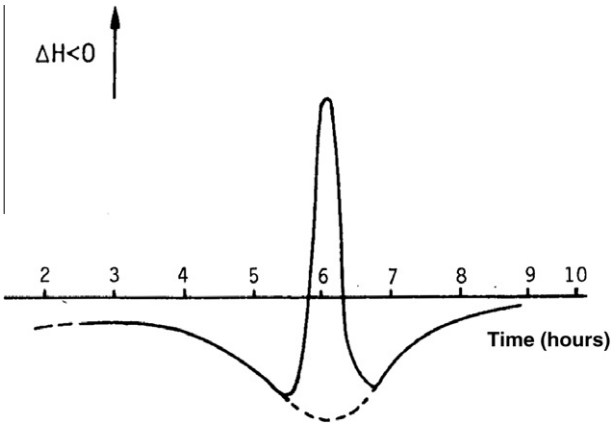

Fig. 2. Microcalorimetric analysis of the conversion of ACP gel into apatite at 25 °C [51]. A broad endothermal peak corresponding to the hydrolysis of PO_4^{3-} ions is superimposed on a narrow exothermal peak corresponding to the crystallisation of apatite.

first event is 'internal' hydrolysis of PO_4^{3-} ions producing HPO_4^{2-} and OH^- ions (see Eq. (1) above) associated with an endothermic reaction giving a rather broad line. The second event is crystallisation, corresponding to a rather narrow exothermic peak. These data can be used to determine the progress of both reactions with time, considered to correspond to the ratio of the area under the different curves at time t versus the overall area of the thermal events. The progress of such reactions can also be followed and determined using chemical analysis of HPO_4^{2-} ions and X-ray diffraction analysis (for apatite content), as presented in Fig. 3, distinguishing the slow hydrolysis reaction and the fast crystallisation process.

The kinetics of the hydrolysis and crystallisation reactions have been shown to follow [51] $a = kt + c$, where a is the progress of the reaction (hydrolysis of PO_4^{3-} or crystallisation), k is a kinetic constant following Arrhenius law and c is a constant depending on the initial state of the precipitate, especially the initial content of HPO_4^{2-}. The initial composition thus appears to be an important parameter in the conversion reaction, one which has often been neglected and may possibly explain most of the divergence between authors.

According to Heughebaert, crystallisation is related to the hydrolysis of PO_4^{3-} and occurs when one-sixth of the PO_4^{3-} has been transformed into HPO_4^{2-}, which also corresponds to occupancy of half of the OH^- sites in the apatite unit cell and the chemical formula [24]:

$$Ca_9(PO_4)_5(HPO_4)(OH).$$

The activation energy of these reactions can then be determined (25.2 kcal mol^{-1}). For the autocatalytic effect experiments by Guégan using apatite seeds have shown that crystallisation spreads from the point where the apatite seeds were introduced [51].

5.3.2. Conversion in aqueous suspensions

Several works have investigated conversion in solution [2,50,52,54] and the effect of factors such as the pH, temperature and the presence of foreign ions.

In a detailed investigation at different pH values (6.8–10) Boskey and Posner [50] showed that the conversion rate was considerably slower at alkaline pH (about 5 h at pH 9). In fact, ATCP is generally precipitated in alkaline media and sometimes even washed with an alkaline (ammonia containing) solution to prevent any alteration during filtration and washing [23,37]. As the pH becomes more acidic the conversion rate becomes faster although some divergence exists, probably related to different experimental conditions and starting materials. Eanes et al. reported a conversion time of 0.3 h at neutral pH and 25 °C, much shorter than the conversion rate observed for gels (6.5–7 h) by Guégan [51]. At acidic pH (3–4) the amorphous phases only exist for minutes and are converted into DCPD instead of apatite. It seems, however, that at very alkaline pH (12.8), the conversion time was again shorter (<1 h) [52].

The hydrolysis of ATCP has been shown to be temperature dependent [50]. It takes ~3 days before crystallisation begins at 10 °C, 2 h at 26 °C and <30 min at 37 °C (pH 8). In the gel form an analogous evolution is observed and at neutral pH it takes ~15 h for complete hydrolysis at 20 °C, whereas at 25 °C the reaction takes only 7 h [51]. At 37 °C hydrolysis is even faster and can be completed in 20 min in a cement paste [55] (see a more detailed presentation in Section 8.2.1).

Several compounds or ions can alter the conversion reaction: mineral ions like Mg^{2+}, Al^{3+} and Ga^{3+}, carbonate and pyrophosphate ions which are present in biological media, several organic molecules which can inhibit or delay conversion and other ions such as fluoride which, in contrast, accelerate conversion [23,54–59].

Several mechanisms have been proposed involving the inhibition of apatite nucleation and growth, the mobilisation of water molecules in gels and disorganisation of the cluster assemblies.

5.3.3. Conversion from dry powders

ACPs are generally in the dry state for different applications in the biomaterial field. Data on the conversion of dry ATCP powders (lyophilised or heated) into apatite in aqueous media seem to differ from those obtained with ATCP gels (Fig. 2) or suspensions; the microcalorimetric curve of lyophilised ACP powder conversion to apatite at 25 °C is presented in Fig. 4 [43]. The endothermal event can no longer or only barely be detected and instead exothermic peaks can be observed, corresponding to rehydration of the powders. The rehydration process occurs in two steps: fast rehydration of the surface of the ATCP spherules, followed by slow rehydration of the inter-cluster space within the spherules. The first rehydration event is not dependent on the temperature of preheating of the ATCP, but the second process is. The limiting step of the conversion reaction of dry powders is the slower second rehydration event.

5.3.4. Conversion mechanisms

Several mechanisms of conversion of ATCP into apatite have been proposed. For Eanes, dissolution–reprecipitation is the major

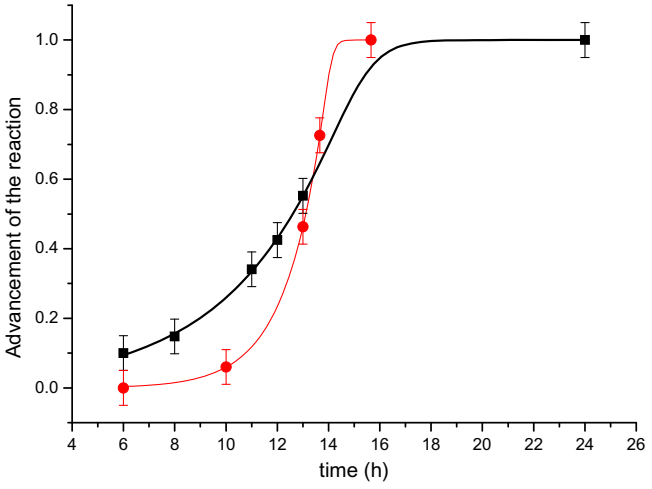

Fig. 3. Conversion of ATCP in gel form. Comparison of the advancement of the hydrolysis of PO_4^{3-} obtained from chemical analysis of HPO_4^{2-} (squares) with the advancement of crystallization obtained by XRD analysis (intensity of the 002 peak of apatite) (circles) [51].

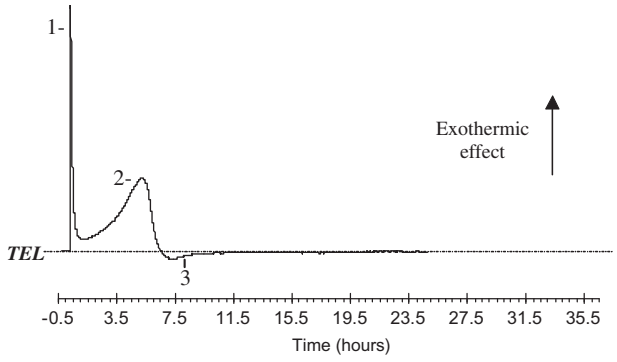

Fig. 4. Microcalorimetric curve of the conversion of lyophilised ACP powder into apatite at 25 °C. Three events can be distinguished: (1) a sharp exothermal peak corresponding to wetting of the powder; (2) a broad exothermic peak corresponding to slow rehydration and crystallisation of the amorphous calcium phosphate; (3) a very small endothermal peak assigned to the hydrolysis of PO_4^{3-} ions [43].

process, supported by TEM observations of crystal nucleation and growth on the spherules of ACP [2]. It has been suggested, based on ionic products in the solution, that at neutral pH OCP is first formed, which is then converted to apatite through a topotactic reaction. This process is in accordance with the Ostwald's step rule. At alkaline pH, however, the conversion of ATCP leads directly to hydroxylated apatite. The OCP intermediate phase does not seem to have been identified under all conversion conditions. This mechanism supposes a change in the Ca/P ratio of the solid, which has to be compensated for either by a change in the Ca/P ratio of the solution (an increased Ca/P ratio relative to the 1.5 initial value) or the simultaneous formation of another solid phase with a higher Ca/P ratio, which could be apatite, however, this phase, which is thermodynamically more stable than OCP, would be favoured. Analyses of the solution at the beginning of conversion generally led to higher Ca/P ratios, in part supporting this mechanism.

Another proposed mechanism is the reorganisation of Posner's clusters to build apatite crystals. It is more and more frequently postulated, with experimental support, that crystals can grow by the accretion of ion clusters formed in solution instead of individual ions reaching the surface [60,61]. As apatite-like Posner's clusters pre-exist in ACP it is conceivable that simple reorganisation of the clusters could lead to apatite crystals, with a minimum activation energy requirement [62]. This process would not change the global chemical composition or the Ca/P ratio as observed, for example, in the conversion process occurring in gels. In addition, the conversion reaction is also observed in water–alcohol media, in which ion dissolution is weak and dissolution–reprecipitation seems improbable. However, even in gels and water–alcohol media conversion into apatite generally involves the formation of HPO_4^{2-} and OH^- ions. Such changes of composition cannot be accounted for by a simple reorganisation process.

The third process, proposed by Puech et al. and Guégan, is surface-mediated transformation [51,63]. The hydrolysis of PO_4^{3-} occurs on the surface of the ATCP agglomerates and leads to the nucleation of apatite, with crystal growth supported by surface ion migration. This process seems consistent with observations of apatite crystal formation, even in the presence of a small amount of water or in water–alcohol media [63]. In addition, when conversion occurs in solution this process is compatible with incorporation into the growing apatite crystals of ions from the solution. Such a conversion process also seems consistent with the observation that rehydration of powdered ATCP is the limiting step in the conversion reaction. As the surface layer has different dissolution properties to the original ATCP precipitate, this conversion mechanism could also be compatible with the observation of a transient phase based on dissolution behaviour but never detected using diffraction techniques.

At this stage, however, the mechanism of conversion has not yet been fully elucidated and it seems reasonable to postulate that competitive processes might occur simultaneously and that their importance depends essentially on the conversion conditions. Once apatite or OCP is nucleated, for example, dissolution–reprecipitation necessarily occurs, although the progress of this reaction could be linked to the solid/solution ratio, the temperature of conversion and the ion diffusion ability of the medium.

6. Characterisation of ACP

6.1. Diffraction techniques

The X-ray diffraction (XRD) pattern of ACP is characterised by several broad diffraction halos, as it can be observed in Fig. 5. Treatment of the XRD data gives radial distribution functions (RDFs), presented in Fig. 6, provides several atomic distances matching the Posner's clusters model. The RDFs of ACP disappear at ~0.95 nm, in contrast to that of well-crystallised compounds such as apatite. This characteristic can be used for the quantitative determination of ACP with a detection limit of ~5% [9,64].

The background level in the XRD pattern is commonly used by ceramicists and polymerists to determine the amorphous fraction of their materials. It is generally considered that what does not correspond to the 'crystalline' phase in diffractograms of CaP materials is 'amorphous'. However, there seems to be some confusion between non-coherent diffraction domains and the existence of a real separated amorphous phase. Some CaPs, especially but not exclusively those with an apatite structure, can exhibit many different ionic substitutions, defects and vacancies which can alter the regularity of the atomic array and cause a considerable increase in the background diffraction patterns without the involvement of any amorphous phase or even amorphous domains. Similarly, in nanocrystals the atom array can be altered close to the surface, leading to amorphous-like domains identified by diffraction techniques or spectroscopic techniques, which should not be confused with the existence of an amorphous phase because they belong to the crystal. Thus, there is agreement that the background level in diffractograms does not really give the amount of ACP phases, but rather represents irregularities in the periodic distribution of the atoms of the apatite crystals.

6.2. Vibrational spectroscopy

Based on Posner's clusters with a S_6 symmetry and group theory analysis, Somrani et al. predicted several bands in the different vibrational domains of the PO_4 groups, which are presented in Table 2 [28,36]. However, these bands are often difficult to distinguish in the FTIR spectrum of ACP (see Fig. 7) because of the band superimposition and broadening characteristic of poorly crystallised and amorphous samples. Resolution enhancement of the spectra has been attempted using mathematical treatment (self-deconvolution) of the FTIR PO_4 bands [36]. The main characteristic of the FTIR and Raman spectra of ACP is a ν_1 PO_4 band shifted to ~950 cm^{-1}, 10 cm^{-1} lower than that of apatite. This characteristic has been used to distinguish ACP phases, however, bands at the same position as those of ACP might also be due to disordered domains arising, for example, from the drying of surface hydrated layers of poorly crystalline apatites. Thus, it seems incorrect to attribute these spectroscopic features to an amorphous phase distinct from nanocrystalline apatite. The quantitative determination of ACP using FTIR and Raman spectroscopy does not seem to have been attempted and, considering the above observations, would seem erroneous in the absence of other investigations providing evidence for the presence of a distinct amorphous phase.

6.3. Differential thermal analysis

These methods are among the most accurate for the quantitative determination of distinct ACP phases in high and low temperature amorphous phases, including mixtures of ACP and other CaP phases [43,49]. The principle is very simple: ACP crystallisation is characterised by a generally rather narrow exothermic peak in the 600–750 °C range. The amount of amorphous phase in a mixture is proportional to the exothermic event. The sensitivity depends on the system considered. For ATCP/HA mixture it is possible to detect 5% ACP (weight ratio). The sensitivity is, however, significantly less for ACP samples containing Mg^{2+} or carbonate ions, due to broadening of the crystallisation peak.

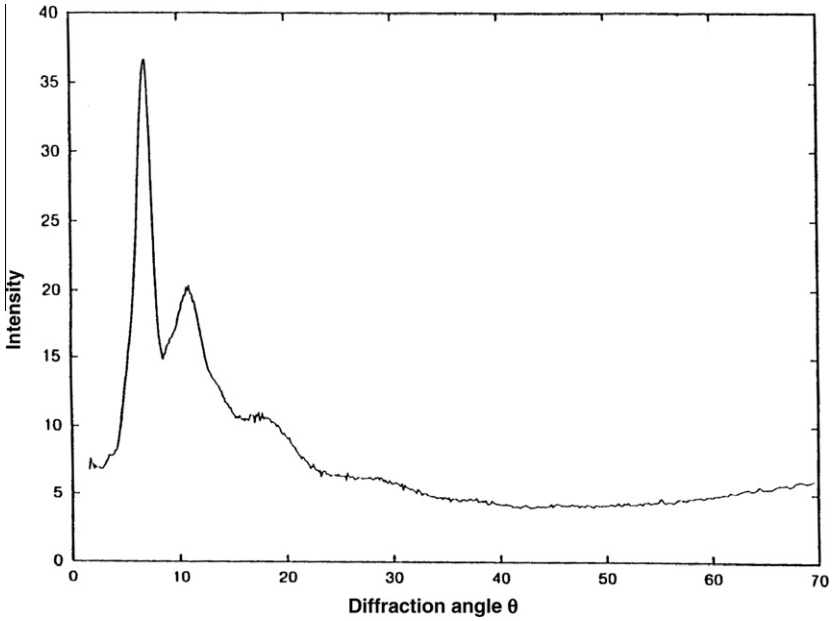

Fig. 5. X-ray diffraction pattern of high temperature amorphous calcium phosphate showing the broad diffraction halos (MoKα).

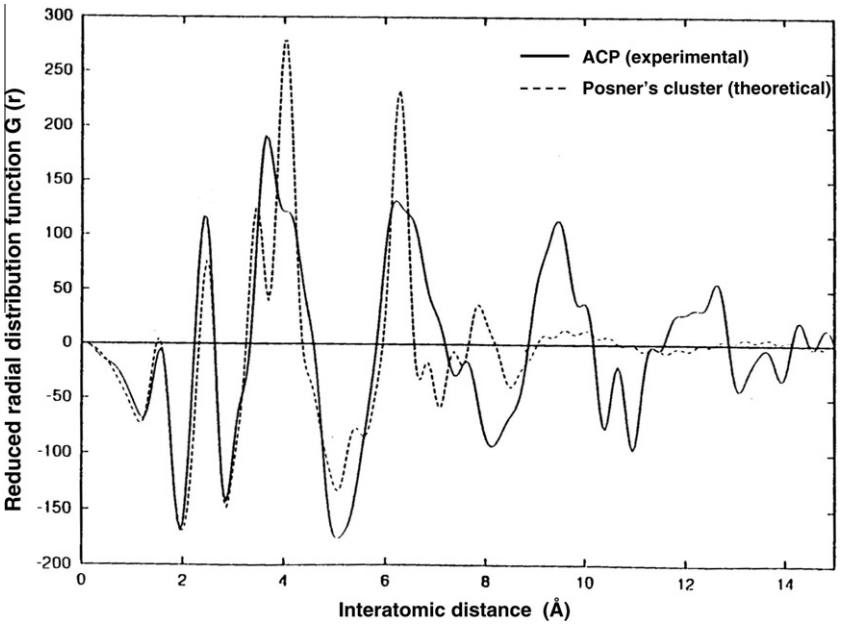

Fig. 6. Experimental radial distribution function (RDF) of a high temperature amorphous calcium phosphate (bold line) compared with the theoretical RDF of Posner's clusters (dotted line) [31]. The size of the experimental clusters appears larger (13 Å) than that of the Posner's clusters.

Table 2
Theoretical spectra of internal vibrations of phosphate ions in Posner's clusters (symmetry S_6).

Spectral domain	Symmetry species	IR and Raman activities
ν_1	A_u, E_u, A_g, E_g	2 IR, 2 Raman
ν_3, ν_4	$3A_u$, $3E_u$, $3A_g$, $3E_g$	6 IR, 6 Raman
ν_2	$2A_u$, $2E_u$, $2A_g$, $2E_g$	4 IR, 4 Raman

6.4. Solid-state NMR

Several MAS-NMR spectra of ACP have been published for ^1H and/or ^{31}P [25,65–67].

The ^{31}P spectra show only one main very broad band at ~3 ppm. relative to H_3PO_4, consistent with a unique phosphate site, as proposed in Posner's model. The position of the very broad band for ACP is close to that for phosphate ions in apatites (2.7 ppm) [68].

The ^1H spectra of ACP reveal the presence of small amounts of HPO_4^{2-} and OH^- ions undetected by spectroscopic techniques (Table 3). It has been suggested that several organised micro-domains, too small and not numerous enough to give a clear diffraction pattern, could exist in ACP, especially apatite-like domains around the OH^- ions and, possibly, water molecules. ^1H NMR seems particularly sensitive in detecting faint changes in ACP during ageing [25].

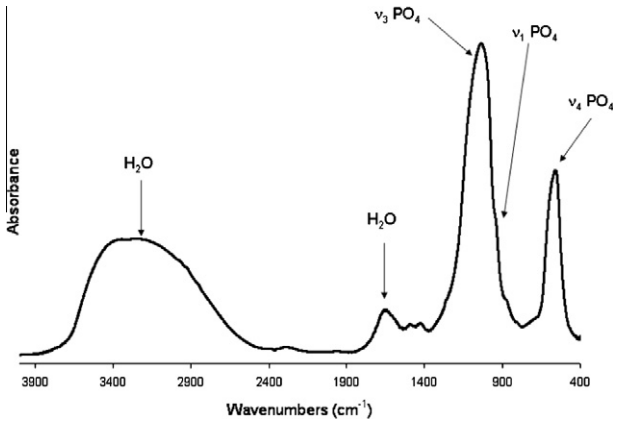

Fig. 7. FTIR spectrum of ACP.

Many other techniques have been used to try to characterise ACP phases.

EXAFS at the K edge of calcium provides spectra particularly consistent with Posner's model [33]. XANES of ATCP at the Ca–K edge gives a specific spectrum (broad singlet) distinct from that of apatite (doublet of the most intense peak) [69]. However, these methods cannot be used for the quantification of ACP phases in mixtures.

7. Synthesis of ACPs

ACPs can be obtained by two main routes: in aqueous medium at low temperature (wet route) or using high energy processing or high temperatures (dry route). Depending on the method of the formation and experimental conditions (solution supersaturation, pH, etc.), the ACP synthesised can exhibit a Ca/P ratio ranging from 1 to 2 or even higher. The methods of synthesis of ACP presented hereafter focus on the most commonly known and used ACPs, i.e. those with a Ca/P ratio of 1.5 or 1.33.

7.1. Wet route synthesis

The wet synthesis route of ACP is based on the double decomposition of a calcium and phosphate salt in aqueous or water–alcohol solutions [23,70,71].

7.1.1. In aqueous medium

ATCP (Ca/P = 1.5) can be prepared by various solution-mediated routes, usually involving rapid mixing and precipitate filtration. Most syntheses are at low or room temperature and involve high supersaturation ratios.

One of the most convenient preparation methods is double decomposition between aqueous solutions of soluble calcium and phosphate salts at ambient temperature and at a pH close to 10 [23,43]. For example, calcium nitrate solution [46.3 g of $Ca(NO_3)_2{\cdot}4H_2O$ dissolved in 550 ml of deionised water containing 40 ml of 28 wt.% ammonia solution] was rapidly poured into stirred ammonium phosphate solution [27.2 g of $(NH_4)_2HPO_4$ dissolved in 1300 ml of deionised water containing 40 ml of 28 wt.% ammonia solution]. Immediately after precipitation the suspension was filtered and washed with 3 l of deionised water containing 15 ml of 28 wt.% ammonia solution and finally lyophilised for 72 h [43].

Storage of ACP powders in a freezer is necessary to prevent conversion and/or phase transformation. Even after drying in a desiccator or lyophilisator ACP contains a significant proportion (10–20%) of tightly bound water molecules [71,72]. Dried ACP can, however, be obtained by heating the powder at 450 °C under vacuum or in air without any discernible differences in the X-ray diffraction patterns before and after treatment.

ACP can be stabilised, especially in industrial processes, by adding a few per cent Mg^{2+}, pyrophosphate and carbonate ions. When heated, pyrophosphate and carbonate ions can react to generate phosphate groups:

$$P_2O_7^{4-} + CO_3^{2-} \rightarrow 2PO_4^{3-} + CO_2 \uparrow \quad (2)$$

Dry, heated ATCP is more stable than unheated ACP and can be stored at room temperature. Generally, ACP is prepared with relatively concentrated solutions and at very high supersaturations and precipitation is immediate. When the level of supersaturation is lower an induction period may occur.

The induction period is highly sensitive to several parameters related to solution composition: a higher initial [Ca] × [P] product or Ca/P atomic ratio and higher temperature or pH, a lower dielectric constant or the presence of Mg^{2+}, $P_2O_7^{4-}$ or CO_3^{2-} ions reduced the time required for induction [4].

The main difficulty encountered in the preparation of ACP is related to its instability and reactivity in solution, which could limit reproducibly producing ACP powders by precipitation. As already mentioned in Section 5.3.2, ACP can be stabilised by the presence of inorganic ions such as Mg^{2+}, $P_2O_7^{4-}$, CO_3^{2-} or organic molecules in the synthesis and/or washing solutions [53,73–75]. For example, ATCP can be prepared under more acidic conditions (pH ∼ 6) and in the presence of magnesium or citrate ions, known to be inhibitors of apatite crystal growth [76,77]. LeGeros et al. observed a synergistic effect of a combination of potent apatite crystal growth inhibitor ions (Mg^{2+}, Zn^{2+}, Fe^{2+}, $P_2O_7^{4-}$ and CO_3^{2-}) on the formation of ACP [78,79].

Li and Weng reported an original method to prepare ACP by precipitation at low temperature (5 °C) by adding polyethylene glycol (PEG) to a calcium solution [80]. They showed that ACP could be stabilised by PEG in the mother solution for more than 18 h at 5 °C and that the Ca/P atomic ratio of the precipitated ACP could be adjusted between 1.33 and 1.50 by controlling the pH and initial Ca/P atomic ratio in solution.

In aqueous media ACP is generally obtained at alkaline pH and the fresh ACP does not contain acid phosphate groups. Under these conditions ACP shows the composition of TCP with an atomic Ca/P ratio close to 1.5 [24]. In acidic or neutral solutions the amorphous phase appears unstable and is rapidly transformed into crystalline CaP phases such as DCPD, OCP or apatite. This evolution can be delayed in water–alcohol solutions to allow ACP with a low Ca/P ratio, containing acidic phosphate groups, to be prepared [81].

7.1.2. In water–alcohol or alcoholic media

The presence of ethanol led to a decrease in the dielectric constant favouring the existence of protonated species such as HPO_4^{2-} [20]. In addition, it has been reported that ethanol stabilises ACP [82].

Table 3
Positions and assignments of the ^1H NMR peaks in ATCPs.

Line positions (ppm)	Attribution
0.2	OH^- ions in apatite environnent
2.3	Apatite-like channel water
5	Adsorbed water
5.9	Interstitial water (inter-clusters)
7.3	Strongly hydrogen bound water
8–11	HPO_4^{2-} acidic protons in different environments
11–14	HPO_4^{2-} acidic protons in different environments
14–17	HPO_4^{2-} acidic protons in different environments

Several preparations have been described [20,81,83]. Rodrigues and Lebugle showed that the presence of ethanol in the precipitation medium influences the composition of the amorphous phase, especially the HPO_4^{2-} content and Ca/P ratio [83], and ACPs with Ca/P ratios close to that of OCP, corresponding to the chemical composition $Ca_8(PO_4)_4(HPO_4)_2$, can be obtained. As for aqueous preparations, preservation of the amorphous phase is only possible if the precipitate is dried by lyophilisation and stored in a freezer. [20].

Layrolle and Lebugle reported an original synthesis route for ACPs using calcium diethoxide [$Ca(OEt)_2$] and orthophosphoric acid (H_3PO_4) precipitated in a dry argon atmosphere [21]. Briefly, $Ca(OEt)_2$ was first prepared in a dry inert atmosphere (argon) by heating refluxed calcium metal in ethanol for 4 h until it disappeared. The orthophosphoric acid solution was then quickly poured into stirred $Ca(OEt)_2$ solution. After centrifugation and removal of the supernatant the powder was dried under vacuum at room temperature overnight before storage under argon at –20 °C to prevent crystallisation or hydrolysis. The authors prepared and fully characterised nanosized ACPs of various compositions [amorphous $CaHPO_4$, $Ca_8(PO_4)_4(HPO_4)_2$ and $Ca_9(PO_4)_6$] in ethanol simply by varying the Ca/P ratio of the reactants (1/1, 8/6 and 9/6); these ACPs had high specific surface areas and reactivities. This ACP sol–gel synthesis method has also recently been used by Fellah et al. to prepare an ACP that can be transformed into β-TCP, HA or HA–β-TCP ceramics after sintering at 1100 °C, depending on the initial Ca/P ratio of the reactants [84].

ACP phases (ACP1 and ACP2) have been observed as intermediate compounds during the ageing of calcium phosphate precipitated in methanol at room temperature, finally leading to nanosized β-TCP formation [85].

Finally, an ACP corresponding to an amorphous OCP has been prepared in 50–50 vol.% water–alcohol medium [81].

7.2. Dry route synthesis

ACP formation can also be prepared by dry methods based on rapid quenching of melted CaPs or low temperature formation of CaPs using ion sputtering. These generally lead to phases with variable compositions, sometimes containing impurities, and are not convenient for the preparation of large amounts of pure ACP. In the absence of other mineral ions than Ca^{2+} and PO_4^{3-} the composition of the amorphous phase formed is analogous to that of anhydrous precipitated ATCP for charge balance reasons [31]. Calcium is often the only cation, but anions other than phosphate, such as O^{2-}, may also be present in the high temperature amorphous phase, producing an increase in the Ca/P ratio (>1.5), especially for ACP in HA plasma sprayed coatings (see Section 8.1.1) [36]. High temperature ACP phases with a lower Ca/P ratio than ATCP can only result from contamination with other cations or anions, such as pyrophosphate.

Recently, Yu et al. reported the synthesis of ACP using a dry mechano-chemical method involving a mixture of DCPD and $Ca(OH)_2$ reactants with a Ca/P ratio of 1.67 [86]. Other authors have shown that prolonged high energy ball milling of β-TCP powder in ethanol or of a ACP and DCPD dry mixture led to amorphous calcium phosphate after 24 h [87,88]. However, there is a non-negligible risk of powder contamination (ball wear) when using this processing route over extended periods of time to obtain amorphous calcium phosphate.

Loher et al. have described a procedure to prepare ACP nanoparticles using a flame spray pyrolysis technique [89]. The precursor solution was prepared with appropriate amounts of calcium-2-ethylhexanoate and tributyl phosphate to produce a Ca/P ratio in solution of between 1.43 and 1.67; doping metals such as zinc or magnesium can also be added to the precursor solution. The precursor mixture is then dispersed in oxygen gas and fed into a methane/oxygen flame: the burning spray of CaP resulted in flame synthesis of ACP nanoparticles. The nanoparticles leave the flame at the top where they are collected on a filter. Fast cooling after formation in the flame prevents crystallisation and leads to ACP nanoparticles (10–50 nm in diameter) with a broad range of compositions, depending on the Ca/P ratio of the precursor solution [89], which after calcination led to dicalcium pyrophosphate (for an initial Ca/P ratio of <1.5) or pure β-TCP (for an initial Ca/P ratio of 1.52) or HA (for calcium-rich precursor) [89]. However, high costs are associated with this ACP synthesis route, mainly due to the high energy flame production and the use of precursors.

8. Uses of ACPs in biomaterials

ACPs are involved in numerous biomaterials in the form of coatings, cements, ceramics and composites (Table 4). The presence of ACP in these biomaterials is in some cases desired and controlled but in other cases, such as plasma sprayed HA coatings, the amount cannot be well controlled. Examples found in the literature will be presented hereafter for each of these kinds of ACP-containing biomaterials.

8.1. ACPs in coatings

Metals are still and will probably for a long time remain the main constituents of load bearing prostheses. Efforts have been made to improve the biological activity of metallic surfaces by coating them with CaP, with the aim of stabilising the bone–prosthesis interface and ensuring early and long-term performance.

ACPs are formed in many coating processes by a low temperature wet route or a high temperature dry route.

8.1.1. ACPs in high temperature and/or high energy coatings

ACPs occur mainly as a co-product in coatings obtained by high temperature and/or high energy processing techniques such as plasma spraying deposition, electrostatic spray deposition (ESD) and pulsed laser deposition (PLD).

Plasma sprayed HA coatings are the most industrially developed CaP coatings. ACPs have been found to play a major role in the mechanical properties of plasma sprayed HA coatings, especially their adhesion to metal surfaces and biological properties [90–93].

The plasma spraying technique is based on the use of plasma flames reaching very high temperatures (5000–20,000 °C) and high velocities that project the HA particles onto the surface to be coated. The individual HA particles are brought to a very high temperature during the short transit time within the flame and are quenched on the metal surface where they adhere. The CaP coating obtained mainly comprises HA, but other crystalline and ACP phases are also present. The amounts of these co-produced phases are related to the conditions of spraying, such as gas flow, which controls the time the HA particles spend in the plasma flame, plasma temperature, nature of the gas, cooling conditions and the distance between the substrate and the flame, along with other determinant factors such as the quality, density, adsorbed water and size of the HA particles [94–97].

ACP is always formed in plasma sprayed coatings of CaP phases. The sprayed HA particles are covered by a fused layer (molten phase) during their transit in the plasma and ACP formation results from rapid quenching of this layer on the surface being coated.

The temperature gradient within the HA particles induces an heterogeneous distribution of the various crystalline and amorphous phases within the particles: a schematic representation of the heterogeneous composition of plasma sprayed HA particles is shown in Fig. 8 [98,99].

Table 4
Amorphous calcium phosphates in biomaterials.

Type of material	Application	Main CaP-related effects
Ionic cements	Bone substitute Dental applications	Active hardening agents Bioresorbable, surface reactivity Provider of Ca^{2+} and PO_4^{3-} ions
Coatings	Coating of metallic prostheses	Biodegradable and reactive coating
Mineral–organic composites	Teeth,enamel remineralisation Bone substitute	Mechanical properties Ca and P release in relation with biological activity

As mentioned in Section 6.1, the presence of an ACP phase is attested to by the existence of a broad band in the X-ray diffraction diagram of a coating (see Fig. 9). This method is recommended by standard NF S 94-067 (Materials for surgical implants – qualitative and quantitative determination of the foreign phases present in calcium phosphate based powders, deposits and ceramics) to quantify the ACP content of plasma sprayed HA coatings and their Ca/P ratios [96–102]. The amount of ACP depends on the coating conditions and can vary between 20 and 60% in commercial coatings.

The longevity of a prosthesis very much depends on the bond at the bone–implant interface and resorption of the coating by the host organism [93]. ACP should be present on the surface coating in only moderate quantities because of its fast dissolution, which promotes fast fixation to bone tissue. However, a minimum amount of ACP is needed to ensure good adhesion of the coating to the metallic prosthesis [92,93,103–105].

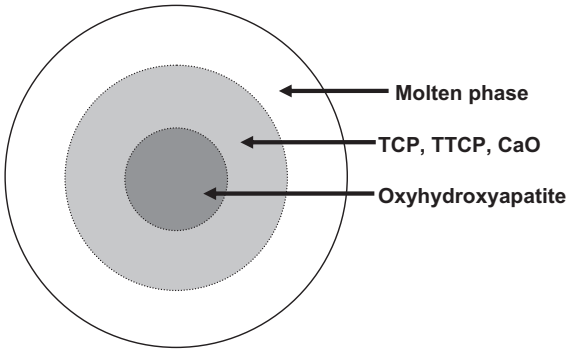

Fig. 8. Effect of temperature gradient on a plasma sprayed HA particle (from [98] with the kind permission of Elsevier).

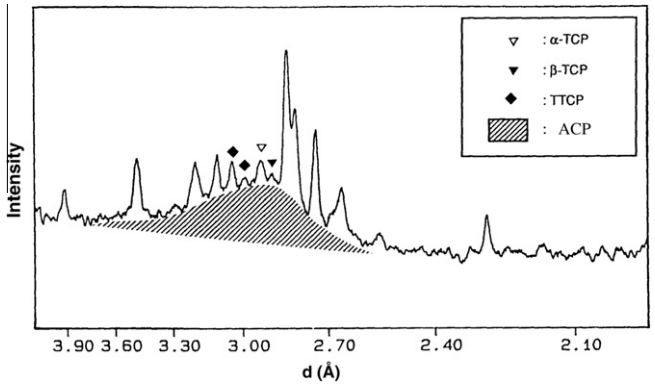

Fig. 9. X-ray diffraction pattern of a plasma sprayed hydroxyapatite coating showing the different amorphous and crystalline calcium phosphate phases [31].

Plasma sprayed coatings can be subjected to moderate temperatures post-treatment to induce ACP crystallisation [105,106]. Other authors have used post-treatment of plasma sprayed HA coatings with water vapour at 125 °C to enhance the conversion of ACP into crystalline HA [107].

The composition of the amorphous phase is rather imprecise and seems to vary according to the authors [98,108]. In fact, the fused layer at the surface of the sprayed particles can partly recrystallise depending on the quenching rate.

The first crystalline phase to form on cooling is calcium oxide, then calcium tetraphosphate and calcium triphosphate and, finally, apatite. Thus the composition of the amorphous phase depends on the respective proportions of the recrystallised phases. Frequently, CaO forms and the amorphous phase shows a Ca/P ratio lower than that of the original apatite phase, although it cannot be lower than 1.5 when only Ca^{2+} and PO_4^{3-} ions are present for electroneutrality reasons.

Other high energy coating techniques have been used to coat metallic substrates with ACP, among which are PLD and ESD [109,110].

In PLD a pulsed laser beam is focused on a rotating HA target in a vacuum chamber under a controlled water vapour atmosphere. Species ejected after each laser pulse reach the surface of the substrate, which can be heated. Several CaP phases can be obtained by varying the deposition parameters, such as the fluence of the beam laser, the water vapour pressure and substrate temperature [109].

A 2 μm thick ACP coating was obtained and tested in vitro in cell culture. After 2 weeks in cell culture bone matrix could be observed covering the sample, but it delaminated after drying of the sample, indicating poor adhesion and most probably dissolution of the ACP coating [109,111].

ACP has been deposited on a titanium substrate using the ESD technique based on the application of a DC voltage (6.3 kV) to generate a high potential difference between the grounded substrate holder and nozzle. A solution precursor (calcium and phosphate in butyl carbitol) and a heated substrate (350 °C) were used by Siebers et al. [110]. After 45 min of deposition and an additional heat treatment at 400 °C a 1–2 μm thick ACP coating was obtained; treatment at higher temperatures led to apatite coatings. The authors concluded that cell differentiation and proliferation on ACP-coated samples was equivalent to that on apatite-coated samples, thereby showing that the crystallinity of a CaP coating deposited by ESD did not affect mineralisation by osteoblast cells. However, we can reasonably suppose that the ACP coating did not remain amorphous during the 24 days of the cell culture experiment at 37 °C and erroneous conclusions could arise from ignoring this possible evolution.

8.1.2. ACPs in low temperature and/or low energy coatings

Recently, research has focused on the development of new coating techniques that can overcome the main drawbacks associated with HA coatings obtained by the plasma spraying technique, i.e. low resorption, particle release and delamination, inhomogeneity of the composition of the coating, no possibility of depositing thermally unstable phases such as ACP, OCP or carbonated apatite analogous to bone mineral and no possibility of including bioactive and/or therapeutic molecules in the coatings. The group of K. de Groot in The Netherlands has active in the setting up and development of biomimetic processes that enable the deposition of CaP phases of biological interest.

Most coatings prepared at low temperatures have been obtained using supersaturated calcium phosphate solutions [for example simulated body fluid (SBF) solution], leading to CaP precipitation, mostly as apatite, onto the surface of metallic substrates

for dental or orthopaedic applications. The simplest way to promote heterogeneous nucleation of ACP on a metallic surface is to control the supersaturation of the solution to adapt the conditions to the nucleation properties of the metallic substrate. After an induction period the metal becomes covered by a layer of CaP.

An amorphous carbonated CaP coating has been prepared at 37 °C and pH 6 after soaking titanium alloy (TA6 V) disks for 24 h in a calcium phosphate supersaturated solution saturated with CO_2 [112,113]. In this process a primary thin ACP coating formed initially, with a carbonated apatite or OCP coating subsequently being deposited on the ACP at 50 °C. It is also possible to associate such coatings with biologically active molecules. A similar biomimetic process involving soaking metallic discs in highly supersaturated SBF solution (SBF × 5) also led to a thin ACP coating, which was subsequently soaked in a supersaturated calcium phosphate solution including an antibiotic (tobramycin). This second step led to a thick carbonated apatite layer containing tobramycin, on the initial thin ACP deposit. The resulting antibiotic-loaded coating was expected to reduce implant-associated infections by local release of this therapeutic agent [114]. This second step led to a thick carbonated apatite coating containing tobramycin on the primary thin ACP deposit.

Nagano et al. also used a biomimetic technique involving a supersaturated calcium phosphate solution at 35 °C to obtain an ACP coating on polyethersulphone plates [115]. It took 12 days to obtain a 20 μm thick ACP coating and 28 days for a 50 μm thick one. They showed that newly formed bone grew in direct contact with the implant as the coating degraded. However, we can expect crystallisation of the ACP as apatite during this very slow deposition process (12–28 days), but the authors did not provide any further details to support this point.

Even if all these biomimetic methods are simple, they have not been adapted to mass production. Indeed, their main drawbacks are related to slow deposition and the need for supersaturated solutions, thereby raising storage and stability problems and making them difficult to control on an industrial scale.

Other low temperature ACP coating techniques can be found in the literature.

An electrochemical deposition method has been used by several authors to prepare a thin film of metastable CaPs [116,117]. This technique is based on water electrolysis, which induces a local increase in pH at the cathode where the metal substrate to be coated is placed. When the electrolysis bath contains a metastable calcium and phosphate solution this pH rise increases the concentration of PO_4^{3-} and local supersaturation of CaP salts. Under these conditions homogeneous precipitation can occur at the cathode.

Using this technique at room temperature, Royer et al. obtained a coating of amorphous calcium phosphate–carbonate with cracks that could be related to the large amount of water associated with the fresh precipitate and the shrinkage that occurred during drying. No cracks were observed when electrolysis was performed at 70 °C, but these conditions favoured the direct deposition of poorly crystalline biomimetic apatite [116].

Roessler et al. showed that ACP can be deposited on titanium using an electrochemical technique at 36 °C and pH 6.4. They controlled the kinetics of ACP transformation to apatite by adjusting the electrochemical parameters, such as current density (−0.5 to −10 mA cm^{-2}) and time of polarisation (5 s–60 min) [117]. This technique opens up the possibility of preparing biomimetic CaP coatings of different solubilities by combining the more soluble amorphous phase with poorly crystalline apatite. Even if characterisation of coating adhesion was not performed in these studies, the main drawbacks of the electrochemical technique are a slow rate of deposition and poor adhesion of the coating to the metal surface.

Dutour Sikiric et al. recently reported a three-step method for preparing a biomimetic organic–inorganic nanocomposite coating involving ACP particles embedded in organic polyelectrolyte multilayers of poly-L-lysine and poly-L-glutamic acid as the first step [118]. The second step is based on the in situ conversion of ACP to OCP or poorly crystalline apatite when immersed in a metastable calcifying solution. Finally, deposition of a final polyelectrolyte multilayer is performed in a third step leading to smoother surfaces which facilitate primary human osteoblast cell adhesion and proliferation. Cell proliferation on nanocomposites with upper polyelectrolyte multilayers was far superior than on any of the individual components and was equivalent to proliferation on the gold standard (plastics).

8.2. ACPs in hydraulic phosphocalcic bone cements

CaP cements have recently received considerable attention in the field of bone substitutes. They form a mouldable paste that can fit the shape of the bone defect perfectly, leading to hardened bioactive materials favouring bone healing. In addition, some are injectable, which enables the use of minimally invasive surgical techniques for their implantation. However, CaP and ACP-based cements show slow resorption properties and low bone regeneration, especially in cranioplasty [119].

The hardening of CaP cements is related to the entanglement of CaP crystals formed by the hydrolysis of more soluble CaP phases or acid–base reaction between two CaP compounds. However, Driessens et al. reported an osteotransductive bone cement made of ACP which set by a sol–gel transition [120].

It was thought that the high reactivity of ACPs in aqueous media, related to their relatively fast hydrolysis to apatite, would make them good candidats as a reactive powder ingredient for bone cements [75,121]. However, the main problems related to the industrial development of an ACP-based biomedical cement are the hydrolysis rate, water content and stability of ACPs. The hydrolysis rate has to be fast enough to allow hardening of the cement (typically between 10 and 30 min). The amount of water is crucial in cements and the high water content of ACP might prevent hardening. In addition, the lack of stability of ACPs might constitute a problem for industrial processing and storage of the dry cement ingredients (solid phase). A solution is to heat ACPs at 450 °C to remove the excess water and stabilise the amorphous phase.

8.2.1. Physico-chemical properties of ACP-based cements

8.2.1.1. ACP–DCPD cements. There is only one ACP-based bone cement currently on the market, namely Biobon® (α-BSM®). This cement comprises a mixture of ACP (ATCP, 50 wt.%) and DCPD (50 wt.%) which is mixed with an appropriate amount of aqueous medium (deionised water or saline) with a liquid to solid ratio of 0.8 ml g^{-1} at room temperature. An injectable paste is obtained which sets in less than 20 min at 37 °C. After hardening it is constituted of nanocrystalline apatite with crystal dimensions close to those of human bone [122]. Other physico-chemical characterisations comfirmed the strong analogy of the end product with bone mineral [123] and this ACP-based cement has been qualified as 'biomimetic' [124].

The setting of this cement was related to the rapid hydrolysis of ACP to apatite, occurring within 20 min after mixing [124]. After setting, slow hydrolysis of DCPD to apatite occurred in a second step lasting up to 48 h after mixing [124]. These results have been confirmed by FTIR spectroscopy and X-ray diffraction analyses (see Figs. 10 and 11, respectively).

In this cement formulation DCPD crystals acted as seeds or templates to facilitate apatite nucleation and crystal growth; a schematic representation of the ACP–DCPD cement setting and hardening involving ACP and DCPD hydrolysis and HA crystallisation is presented in Fig. 12. The existence of an epitaxial relationship between DCPD and apatite most likely favoured the

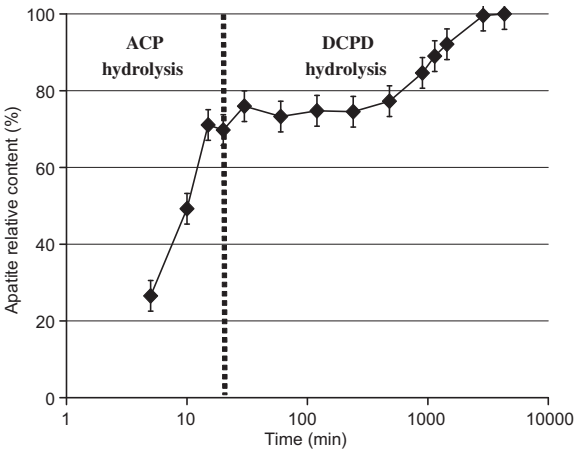

Fig. 10. Conversion of ACP and DCPD into apatite as a function of time (per cent apatite content) determined by mathematical decomposition of the ν_4 PO_4 band obtained by FTIR spectroscopy analysis. During the first 20 min a rapid increase is assigned to the rapid hydrolysis of ACP at 37 °C, corresponding to the hardening time. A slow increase is assigned to the slow hydrolysis of DCPD (from [124] with the kind permission of Trans Tech Publications).

development of apatite crystals from the unstable amorphous phase [125]. The setting and hardening of this ACP-based cement is related to a conversion reaction strongly dependent on temperature. The paste does not harden at room temperature and body temperature is needed for the setting reaction to begin and develop. This characteristic is an interesting advantage allowing an extended working time for the paste at room temperature and linking setting to implantation. The dependence of hardening on paste temperature can be clearly seen in Fig. 13, which shows the evolution at different temperatures of the load required for a 1 mm diameter needle to penetrate 2 mm into the cement paste [123].

Tofighi and Palazzolo have shown that mechano-chemical grinding of the solid phase comprising DCPD and ACP modified the powder characteristics [126]. The particle size of ACP and DCPD was reduced within the first 2 h and DCPD became completely amorphous after 24 h of co-grinding with ACP. Under these conditions the kinetics of hardening of the cement were faster: the setting time was 9 min when using 1 h milled powders and 3 min when using 24 h milled powders (the setting time of the reference

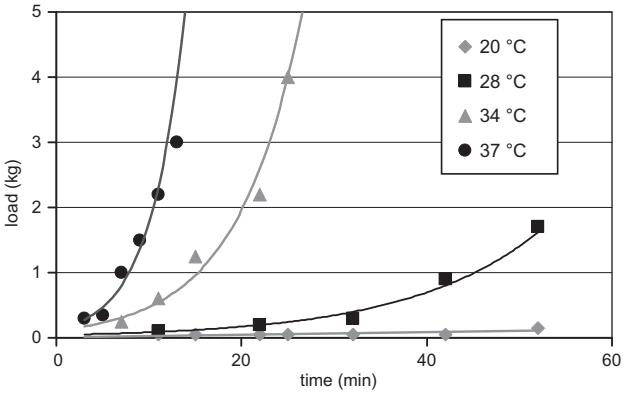

Fig. 13. Paste setting and hardening as a function of time at different temperatures. The values represent the load (kg) required to penetrate 2 mm into the paste with a 1 mm diameter blunt ended needle [123].

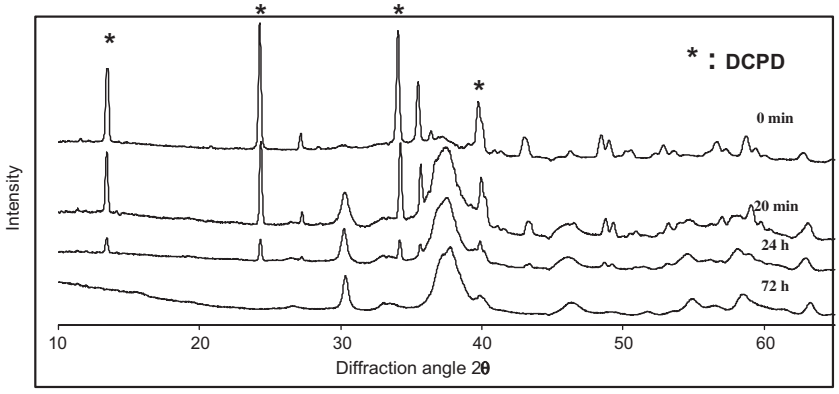

Fig. 11. X-ray diffraction diagrams (CoKα) of the cement during setting. The apatitic phase forms rapidly in the first 20 mi, corresponding to the hardening time. DCPD hydrolysis lasts longer and this phase is still observed after 24 h (from [124] with the kind permission of Trans Tech Publications).

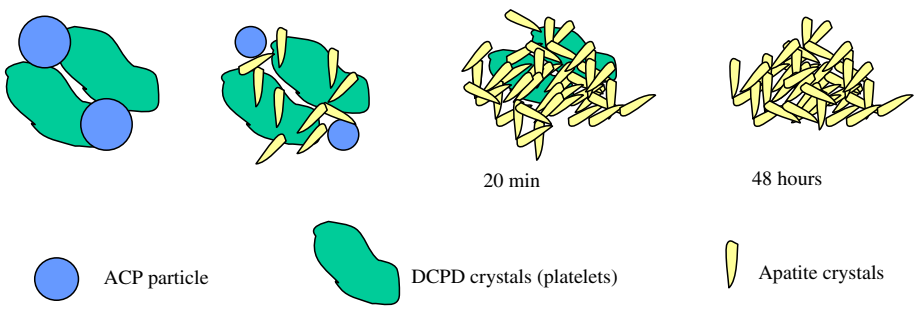

Fig. 12. Schematic representation of the setting reaction of ACP–DCPD cements (α-BSM®, Etex Corp.) involving rapid hydrolysis of ACP into apatite during the first 20 min after paste preparation. The hydrolysis of DCPD into apatite lasts longer (48 h).

cement was 20 min). Co-grinding the powders also improved the mechanical properties of the cement: 50 MPa when using 10 h milled powders compared with 2.6 MPa for the reference cement (unground powders).

Another way to improve the mechanical properties of ACP-containing cements is based on the addition of water soluble polymers to the cement paste. Mickiewicz et al. incorporated polyelectrolytes, polyethylene oxide and bovine serum albumin (BSA) into a commercial ACP-based cement α-BSM®) [127]. Polycation and BSA addition led to increases in cement compressive strength of six times and double, respectively, that of the reference cement. These results correlated with the reduction in apatite crystallite dimensions because of additive adsorption inhibiting crystal growth.

Several authors have studied modified formulations of this ACP-based cement, including additives with a view to improving and controlling other properties, such as injectability [127,128]. In particular, setting time and injectability can be optimised for different requirements by controlling the amount and nature of additives.

For example, the injectability of the paste can be improved by preparing the paste with disodium phosphate solution as the liquid phase, whereas the use of PEG, glycerine or citric acid solutions have been shown to decrease the injectability of the paste and shorten its setting time [127]. A compromise between appropriate injectability and a sufficiently short setting time of ACP + DCPD cements always needs to be found to move towards minimally invasive surgical procedures and avoid cement washout, a phenomenon which generally occurs when the paste is in contact with biological fluids. Wang et al. have studied the effect of introducing natural macromolecules (chitosan, alginate and starch) as anti-washout agents in ACP + DCPD formulations [128].

Owing to its biologically positive effects, especially on osteoblast cells, the introduction of strontium into ACP-based cements has been studied by precipitating ACP by double decomposition using a cationic solution containing strontium and calcium ions [130]. Another way to introduce strontium into a cement is by dry grinding strontium carbonate with the ACP–DCPD solid phase [129]. A positive synergistic effect of the presence of $SrCO_3$ has been demonstrated on several properties of the cement, such as an improvement in injectability of the paste, X-ray contrast (radio-opacity) and mechanical properties of the hardened product [129].

Recently, Yu et al. proposed an original ACP-based cement formulation including mechano-activated β-cyclodextrins (CD)–ACP and DCPD [86]. The mechano-activation of β-CD induced the formation of hydrogen bonds between β-CD macromolecules, which led to an increase in intermolecular interactions in the presence of water. This could lead to a film of β-CD on the surface of the cement in contact with the aqueous medium without affecting the setting reaction, beginning by the hydration of ACP–CD and DCPD in the bulk of the cement. These authors have taken advantage of this property to confer good anti-washout properties on an ACP-based cement [86].

Speirs et al. have shown that a mixture of α-BSM® cement and morsellised bone is a promising composite with improved clinical results in revision hip arthroplasty [131].

8.2.1.2. ACP–anhydrous dicalcium phosphate (DCPA) cements. An ACP–DCPA cement with controlled crystallinity was proposed by Wang et al. [132]. It consisted of a mixture of ACP and DCPA (50 wt.%) with partially crystallised calcium phosphate (PCCP). The latter compound was obtained by heating probably wet ACP for 2 h at 450 °C. The ratio of ACPto PCCP enabled control of the crystallinity of the HA formed after setting and hardening and the resorption properties of the cement. As in the case of the ACP–DCPD cement, in the ACP–PCCP–DCPA cement the PCCP particles acted as seeds to facilitate apatite nucleation and crystal growth during setting. Consequently, a decrease in the setting time was observed when the proportion of PCCP in the cement formulation was increased.

8.2.1.3. Other ACP-containing cements. Van den Vreken et al. studied the influence of ACP introduction on two phosphocalcic cement formulations: an α-TCP-based cement and a TTCP–MCPM-based cement. They also prepared a pure ACP cement as a reference [133]. The authors showed that the addition of ACP to these cement formulations led to a decrease in the crystallinity of the end product (apatite), the setting time and the mechanical properties. They also observed poor cell viability on the pure ACP cement.

As already mentioned in Section 7.2, Gbureck et al. presented an original apatite cement system based on mechanically activated β-TCP [88]. As already mentioned, prolonged milling treatment (24 h) of crystalline β-TCP in ethanol induced a crystalline to ATCP partial transformation. This partly ATCP powder led to a cement which set within 5 min when mixed with sodium hydrogen phosphate (2.5%) solution. An increase in milling time resulted in a decrease in initial setting time and was attributed to the increase in solubility of mechanically activated β-TCP. The authors pointed out that after prolonged mechanical activation particle size was no longer reduced but the milling energy induced an increase in defects within the crystal lattice and β-TCP thereby became partly amorphous.

Brunner et al. studied several TCP-based CaP cements using different TCP crystalline (α-TCP and β-TCP) and/or amorphous phases with powders of varying particle sizes as the starting materials [134]. Cements prepared with pure nanosized ATCP particles showed high reactivity and setting because of their rapid conversion (in 30 min) into highly porous calcium-deficient HA with a high specific surface area. Interestingly, the setting time was less than 12 min when using ATCP particles of 25–60 nm size, but the mechanical properties needed to be improved [135].

Mixtures of ATCP and α-TCP were been investigated by Brunner et al., using nanosized and microsized milled reactive powders and sodium phosphate buffer as the liquid phase [135].

The incorporation of ACP doped with ions of biological interest (Mg^{2+}, Zn^{2+} or F^-) in a cement formulation including α-TCP, MCPM and β-TCP–HA granules was studied by Julien et al. [136]. Even if the role of ACP in this system was not clearly explained by the authors, the cement including ACP doped with fluoride showed a shorter setting time and greater compressive strength than cements including ACP doped with zinc or magnesium.

8.2.2. ACP-based cements as drug delivery systems

Resorbable cements are attractive biomaterials as drug delivery systems for antibiotics or growth factors for orthopaedic and dental applications [137]. The association of rh-BMP-2, a protein known to play a crucial role in the growth and regeneration of bone tissue, with the injectable ACP-based cement α-BSM® has been shown to accelerate bone healing when injected in vivo [138–140].

The association of the growth factor rh-BMP-2 with an ACP–PCCP–DCPA cement has been shown to confer better early osteoconductive and osteoinductive properties on the cement [132].

8.3. ACPs in ceramics

The consolidation of ATCP has been investigated using cold axial compression (P = 400 MPa) or low temperature axial compression (400 MPa and T = 200 °C for 24 h) [122]. No change in composition and structure of the ACP was detected after each process. However, the mechanical properties of the prepared ACP ceramics were poor: in the case of cold axial compression the com-

pressive strength reached 21 MPa and the apparent density was ~1.75, while the samples preheated at 200 °C were not tested because they were too friable.

The possibility of preparing dense ceramics of ATCP was studied by Drouet et al. using spark plasma sintering (SPS) at temperatures ranging from 150 to 200 °C; the SPS processing lasted less than 15 min [141]. These low temperature conditions are more adapted to the processing of less stable phases such as ACP compared with the experimental conditions in conventional sintering. Physicochemical characterisation of the prepared consolidated ceramics indicated crystallisation of the initial ACP powder to an apatitic phase with no other detectable crystalline phase. The microstructure of the prepared consolidated disks exhibited a large intergranular porosity, which could explain the poor mechanical strength observed (Brazilian test, $\sigma = 5$ MPa for 6 min at 150 °C). However, an increase in the SPS processing time to 13 min led to a tensile stress of $\sigma = 12$ MPa, indicating the possibility of improving the mechanical resistance of consolidated disks arising from SPS treatment of ATCP.

8.4. ACPs in composite biomaterials

Several composite biomaterials including ACPs have been studied and used, especially for dental applications. ACPs are involved as a filler in ionomer cements (resins) used to fill cavities or as colloidal suspensions in toothpaste, chewing gum or mouthwash used for the remineralisation of carious lesions and/or to prevent tooth demineralisation [142–149]. These composites take advantage of the ability of ACP to release calcium and phosphate ions, especially in the acidic oral environment. These ions can take part in remineralisation of enamel [147,150,151].

ACP can be incorporated into orthodontic adhesives, crown and bridge adhesives, pit and fissure sealants and restorative composite resin. In these composite materials the ACP used is often Zr-hybridised ACP or sometimes Si-hybridised ACP. As already mentioned in the Sections 5.3.2 and 7.1.1, the presence of certain ions such as Zr^{2+} or SiO_4^{4-} in ACP has been shown to delay its conversion to apatite [152].

Polymer resins used as a matrix are photo-activated methacrylate resins, for example mixtures of ethoxylated bisphenol A dimethacrylate (EBPADMA), triethylene glycol dimethacrylate (TEGDMA), hydroxyethyl methacrylate (HEMA) and methacryloyloxyethyl phthalate (MEP) [151].

Several authors have shown that milling zirconia-hybridised ACP improved the initial mechanical resistance of the composite owing to a better interface and dispersion of ACP within the resin matrix related to the desagglomeration and decrease in ACP particle size [153–155]. The mechanical response of milled ACP composites when immersed in simulated saliva solution was closer to that of glass-reinforced composites, and the authors observed an increase in the incidence of a failure mode consistent with stronger adhesion of milled ACP composites to dentin [154].

Casein phosphopeptide-stabilised ACP nanocomplexes and fluoride-containing toothpastes and mouthwashes have demonstrated anticariogenic activity in vitro and in vivo [147,150,156].

9. Conclusion

ACPs offer a wide variety of compositions that can be found in living organisms (especially invertebrates) or synthesised either under biomimetic conditions or using high temperature/high energy techniques. ACP is easily converted into poorly crystalline apatite analogous to bone mineral crystals and advantage can be taken of its high reactivity to prepare bioactive biomaterials. ACP is involved as a transient or constitutive phase in several commercial substitute bone materials, such as plasma sprayed coatings on metal prostheses and injectable cements for orthopaedic applications. ACP is also used for dental applications as a filler in ionomer cements to fill cavities or as a colloidal suspension in toothpastes, chewing gums or mouthwashes to promote remineralisation of carious lesions and/or to prevent tooth demineralisation.

Even if the instability of ACPs raises problems for mass production, storage and processing that limit the development of ACP-based biomaterials, the reactivity of ACP should certainly be further exploited, especially to prepare coatings with enhanced adhesion and bioactivity. In addition, we can take advantage of its ease of assimilation in vivo to prepare composites with high remineralising potential or drug carriers, even if its adsorption properties are not well known.

Appendix. Figures with essential colour discrimination

Certain figures in this article, particularly Figures 1, 3 and 12, are difficult to interpret in black and white. The full colour images can be found in the on-line version, at doi:10.1016/j.actbio.2010.02.017.

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