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Alkali ion substituted calcium phosphate cement formation from mechanically activated reactants

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Potassium and sodium containing nanoapatite cements were produced from $Ca_2KNa(PO_4)_2$ by prolonged high energy ball milling of the compound for up to 24 h. This mechanical treatment resulted in the decrease of the crystal size and a partial amorphisation of the cement reactant as shown by X-ray diffraction analysis and the appearance of strong exothermic peaks in differential scanning calorimetry measurements. The pH of water saturated with $Ca_2KNa(PO_4)_2$ was 12.5 when the material was mechanically activated but was only 9.5 for the untreated compound suggesting an increase in solubility following milling. The cements set following mixing with a 2.5% Na_2HPO_4 solution in clinically acceptable times between 5–12 min and showed compressive strengths of up to 11 MPa after 24 h setting. The strong alkaline pH value of the cements may provide antimicrobial potential for an application in dentistry as pulp capping agents or cavity liners or for the treatment of infected bone sites.

1. Introduction

The Ca/P ratio of apatitic precipitates in calcium phosphate cements (CPC) is normally in the range between 1.5–1.67 when α-TCP or TTCP /DCPA based cement formulations are used [1–3]. Higher Ca/P ratios with the aim of an enhanced cement solubility could be realised by incorporation of CO_3^{2-} ions into the crystal lattice [4]. Recently it was shown by Driessens *et al.* [5, 6] that nanoapatites can be precipitated in a much broader range $0.8 < Ca/P < 1.5$ by the use of potassium and sodium containing compounds as cement ingredients, like $CaKPO_4$ (CPP) or $Ca_2KNa(PO_4)_2$ (CPSP). These materials are of interest as bone substitutes due to their higher solubility and hence resorption ability compared to normal apatites. However, setting of these described cement systems could be only achieved in combination with MCPA or α-TCP as further cement reactants [5, 6]. Single component systems of CPSP have not yet been described in literature, probably because of the low reactivity of the compounds. The reactivity of cement reactants is altered by the specific surface area of the compounds, which is adjusted by milling the materials in liquid or solid phase [7, 8]. Another mechanism involves changes of the crystallinity of calcium phosphate compounds during prolonged milling, which leads to a partial amorphisation of the compounds and therefore a higher reactivity. This effect has recently been investigated for the reactivity and cement formation of ß-tricalcium phosphate and tetracalcium phosphate [9, 10].

The aim of this work was to increase the reactivity of the alkali ion substituted calcium phosphate $Ca_2KNa(PO_4)_2$ to a point where single component cement systems could be achieved. In this paper we demonstrated that high energy ball milling of this compound resulted in a partial phase transformation to the amorphous state and a strong increase of the formation enthalpy. The setting reaction and mechanical performance of mechanically activated CPSP as single component cement with a Na_2HPO_4 solution was examined.

2. Materials and methods

Calcium potassium sodium phosphate ($Ca_2(K,Na)(PO_4)_2$) was synthesised by heating a mixture of monetite (DCPA; Mallinckrodt Baker, Griesham, Germany), potassium carbonate and sodium carbonate (both Merck, Darmstadt, Germany) in a 4:1:1 molar ratio to 1050 °C for 24 h followed by cooling to room temperature in a desiccator. The sintered cake was crushed with pestle and mortar and passed through a 355 μm sieve. Milling of CPSP for up to 24 h was performed in a planetary ball mill (PM400 Retsch, Germany, diameter: 400 mm unidirectionally) at 250 rpm with 500 ml agate jars, 200 agate balls (10 mm) and a load of 75 g powder and 125 ml ethanol (99.9%), per jar. The ground powder was dried in a vacuum oven at 60 °C. Particle size distributions were determined using a laser particle size analysis (L300, Horiba, Kyoto, Japan) after dispersing 100 mg of the powder particles in 200 ml

isopropanol by applying ultrasound for 15 min. Differential scanning calorimetry (DSC) and thermogravimetric measurements (TG) were performed (Model STA 409, Netsch, Germany) at a heating rate of 10 °C/min up to 1100 °C.

Single component cement was produced by the mixture of ground powders and 2.5 wt% Na_2HPO_4 solution at a powder mass to liquid volume ratio (PLR) of 2–3 g/ml. Samples for mechanical testing were prepared by mixing 800 mg of powder with the required liquid volume in a nitrile rubber mixing container on a vibratory shaker for 15 s. The cement paste was transferred in stainless steel cylindrical molds (6 mm in diameter), both ends were closed with suitable stainless steel plungers and pre-compressed by means of a cantilever device described in literature [11] at a pressure of 9 MPa for 5 s, followed by a constant load of 0.7 MPa. After storing the whole apparatus for 2 h in a 37 °C/100% humidity box, the specimens were removed from the molds and stored in saturated calcium phosphate solution for additional 22 h. Cement dimensions were 12 mm in length and 6 mm diameter. Samples ($n = 9$ per condition) were tested in compression at a crosshead speed of 1 mm/min using a static mechanical testing machine *Zwick 1440* (Zwick, Ulm, Germany) and a 5 kN load cell. The fracture surfaces were subsequently examined using a scanning electron microscope (DSM 940, Zeiss, Oberkochen, Germany). Cement specimens were gold sputtered and then images taken in high vacuum mode at an accelerating voltage of 10 kV. The initial setting time of the cements was measured in a humidity chamber at 37 °C and >90% humidity using the Gilmore needle test with a needle of 113.98 g and 2.117 mm diameter according to ASTM standard [12]. The pH-values of cement pastes were measured over a period of 2 h at a powder to liquid ratio of 2 g/ml using a cut-in pH electrode (Mettler-Toledo, Germany).

X-ray diffraction patterns of the sintered raw materials, mechanically activated compounds and set cements were recorded on a diffractometer D5005 (Siemens, Karlsruhe, Germany). Data were collected from 2θ = 20–40° with a step size of 0.02°. The strut densities of the cement, i.e. the densities of the solid cement products, were found using helium pycnometry at room temperature (22 °C) (Accupyc 1330, Micromeritics, UK). Cement samples were intensively crushed with mortar and pestle prior to measurement to minimize errors caused by the presence of closed porosity. The average densities of three cement samples were calculated using 10 purges and 10 measurements. These data combined with mass and dimension measurements at room temperature enabled calculation of relative porosity of the cement.

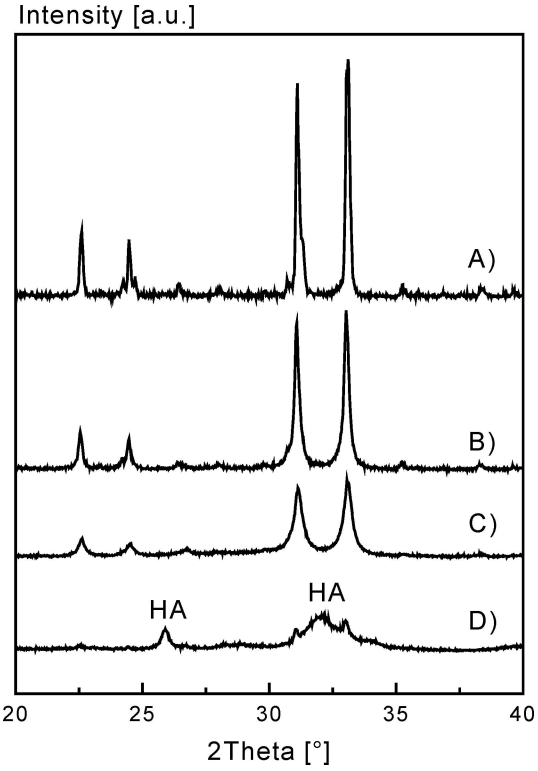

Figure 1 X-ray diffraction patterns of (A) sintered $Ca_2(K,Na)(PO_4)_2$, (B) 4 h grinding, (C) 24 h grinding and (D) 24 h grinding and setting with 2.5% Na_2HPO_4 for 24 h.

3. Results

High energy ball milling of $Ca_2(K,Na)(PO_4)_2$ over a period of 24 h led to a decrease of the medium particle size d_{50} from 18 μm (unmilled) to 0.8 μm (24 h milled) as indicated in Table I. At the same time, it was found that a loss of crystallinity of the CPSP occurred as grinding time increased, as it can be seen by a decrease of the X-ray reflection peak intensity and a broadening of the peaks (Fig. 1(A)–(C)). Differential scanning calorimetry (DSC) patterns of the raw and ground materials (Fig. 2) showed the appearance of broad and strong exothermic peaks between 500–900 °C after grinding the material, which indicated an increase of the formation enthalpy of the compound by ball milling.

While the raw material showed nearly no setting reaction with an aqueous phase, prolonged grinding of the CPSP resulted in a hardening of cement pastes and nearly complete conversion to nanocrystalline hydroxyapatite (Fig. 1(D)) with only a small amount of unreacted $Ca_2(K,Na)(PO_4)_2$ remaining. Compressive strengths were found to increase with milling time from 5 MPa (1 h grinding) up to 11 MPa (24 h grinding) after 24 h setting and initial setting times were in the range between 5–6 min (Table I). The porosity of the

TABLE I Particle size, compressive strength and setting times of $Ca_2(K,Na)(PO_4)_2$ cements after 24 h setting at 37 °C

Grinding time (h)	Particle size size d_{50} (μm)	Compressive strength (MPa)	PLR_{max} (plastic limit) (g/ml)	Setting time (min) at PLR 2.0 (g/ml)	Setting time (min) at PLR_{max}
unmilled	17.94	no setting	–	–	–
1	3.32	5.2 ± 1.4	3.0	12.5	6
4	2.12	7.2 ± 1.4	2.5	8	5
24	0.80	10.9 ± 2.9	2.0	5	5

TABLE II Apparent densities, strut densities and porosity of $Ca_2(K,Na)(PO_4)_2$ cements and strut density of cement reactants determined at room temperature.

Grinding time (h)	Apparent density (g/cm^3)	Strut density of set cement (g/cm^3)	Porosity cements (%)	Strut density of cement reactants (g/cm^3)
–	–	–	–	2.908 ± 0.004
1	1.523 ± 0.057	2.904 ± 0.002	47.6	2.977 ± 0.003
4	1.301 ± 0.040	2.661 ± 0.003	51.1	2.891 ± 0.003
24	1.251 ± 0.032	2.741 ± 0.003	54.3	2.724 ± 0.004

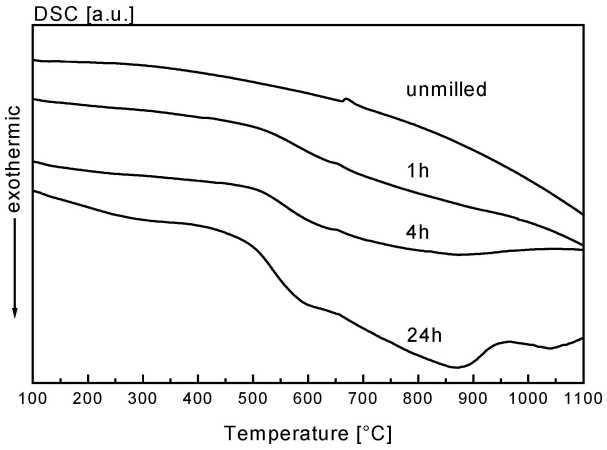

Figure 2 DSC patterns of $Ca_2(K,Na)(PO_4)_2$; (A) sintered raw material, (B) 1 h ground, (C) 4 h ground and (D) 24 h ground in ethanol.

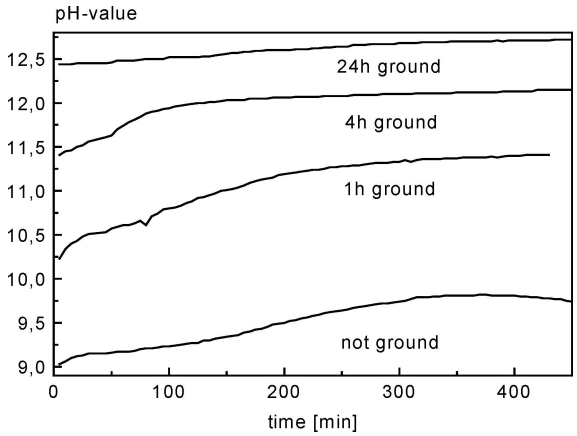

Figure 3 pH-values in $Ca_2(K,Na)(PO_4)_2$ cement pastes after various grinding times during setting over a period of 2 h.

cements increased from 47% (1 h ground) to nearly 54% (24 h ground) due to the higher amount of cement liquid necessary for forming a workable cement paste (Table II). At the same time, the pH-value of cement slurries was strongly affected by prolonged ball milling. The pH of sintered raw material mixed with a near neutral Na_2HPO_4 solution was increased slightly from 9 to 9.5 after two hours, however mechanical activation by grinding subsequently increased the pH-values of the cement pastes to nearly 12, demonstrating that the OH^- ion concentration formed by the hydrolysis reaction of $Ca_2(K,Na)(PO_4)_2$ was increased by more than two orders of magnitude (Fig. 3). Prolonged grinding also affected the morphology of the hardened cement matrix. While the 1 h ground cement showed a course textured microstructure with embedded particles in the range 10–20 μm (Fig. 4(a)), subsequent milling of the cement reactants resulted in a finer microporous cement

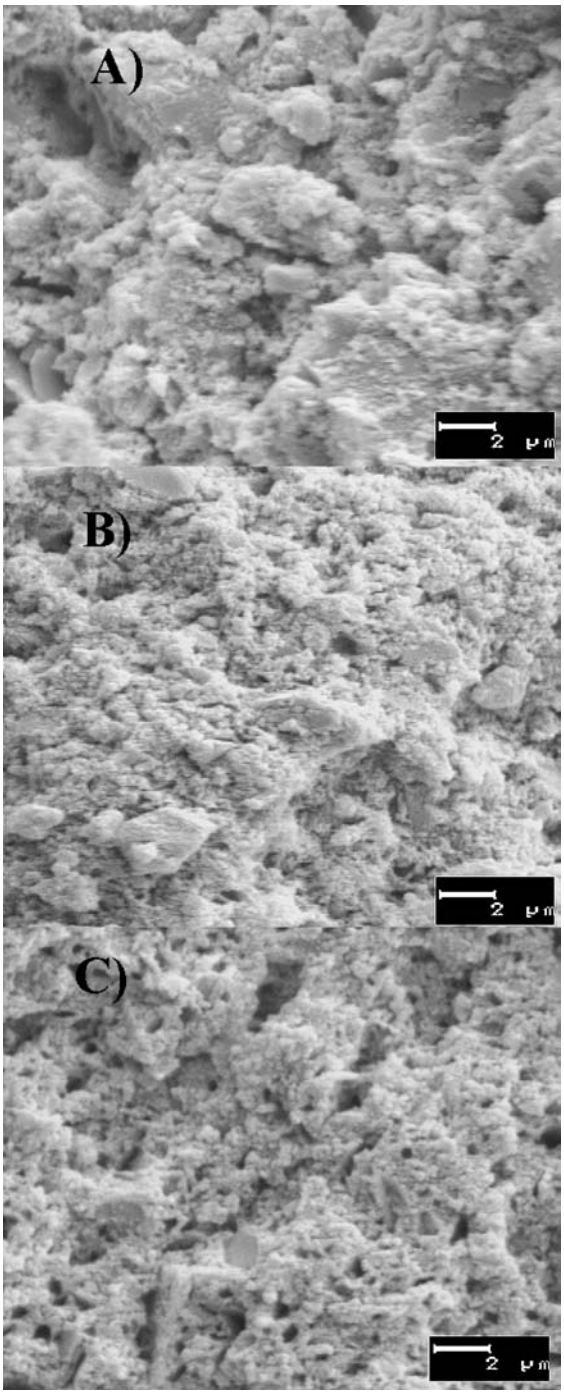

Figure 4 SEM micrographs of $Ca_2(K,Na)(PO_4)_2$ cements after 24 h setting with 2.5% Na_2HPO_4; (A) 1 h ground, (B) 4 h ground and (C) 24 h ground in ethanol.

microstructure with pore sizes in the sub-micron range (Fig. 4(c)).

The stability of mechanical properties was studied by immersing the 24 h ground cement samples in water

TABLE III Compressive strengths of cement samples made from 24 h ground $Ca_2(K,Na)(PO_4)_2$ after immersion in water for up to 28d

Immersion time	1 d	3 d	8 d	14 d	28 d
Compressive strength (MPa)	10.9 ± 2.9	14.9 ± 3.2	10.7 ± 1.8	7.2 ± 1.0	5.2 ± 1.3

for up to 28d (Table III). After an initial increase of the strength to nearly 15 MPa after 3d, compressive strength values decreased continuously to 5 MPa after 28d immersion.

4. Discussion

Apatitic calcium phosphate cements are widely used as bone substitute materials in craniofacial and maxillofacial surgery due to their excellent biocompatibility in hard and soft tissue contact [13, 14]. However, they show only a slow surface osteoclastic resorption due to the thermodynamic stability and low solubility of apatite under physiological conditions. A higher resorption ability should be obtained when substituted apatites by incorporation of carbonate or alkali ions are used. Ion substitution in calcium phosphate cements has been performed by adding calcium or sodium carbonate to the cement powder/liquid [4] or by the use of alkali substituted calcium phosphates as cement reactants [5]. However, in the latter case, the compounds had to be used in conjunction with MCPM and/or α-TCP to obtain setting cement systems, because the hydrolysis reaction of highly crystalline CPSP to potassium and sodium containing apatite according to Equation (1) is very slow:

$$\begin{aligned} &5Ca_2KNa(PO_4)_2 + 2H_2O \\ &\quad \rightarrow 2Ca_5K_2Na_2(HPO_4)(PO_4)_4(H_2O) \\ &\qquad + Na^+ + K^+ + 2OH^- \end{aligned} \tag{1}$$

Here we showed, that the rate of hydrolysis of CPSP as a single cement component could be increased by prolonged high energy ball milling of the particles. The grinding regime did not only affect the particle size and hence the specific surface area of the compound, but also led to microstructural changes of the CPSP in the solid state. X-ray diffraction patterns of the ground materials indicated both a broadening of the reflection peaks and a decrease of the total peak intensity. The first effect is related to a reduction of the primary crystal size, which could be estimated by using the Scherrer equation for spherical particles:

$$d \approx \frac{0.9 \cdot \lambda}{\beta \cdot \cos(\theta)} \tag{2}$$

where d is the crystal diameter, λ is the wavelength, θ is the diffraction angle and β is the peak width at the half maximum. This calculation for the two strongest reflection peaks at $2\theta = 31.1^\circ$ and $2\theta = 33.2^\circ$ resulted in a medium crystal size of 87.7 nm (60.0 nm) for the sintered raw material, which decreased to 27.5 nm (27.4 nm) after 24 h ball milling. The second effect of peak intensity reduction is related to the formation of amorphous domains within the particles with crystal sizes below 1 nm. The loss of crystallinity was estimated from the peak areas of the two reflection peaks. The peak area is decreasing by 36–39% and it can be concluded that the loss of crystallinity and the formation of amorphous material is in the same order of magnitude. This mechanically induced amorphisation process alters the solubility of the material which could be observed in the pH development of cement pastes, since the concentration of the reaction product OH^- was increased by more than two orders of magnitude.

However, the cements provided only relatively weak compressive strength (maximum mean value = 11 MPa) compared to other cement matrices, e.g. α-TCP or TTCP/DCPA, where strengths of up to 80–130 MPa have been reported recently [15, 16] under the same measurement conditions. This is probably due to the higher porosity of the CPSP cements since strength is an inverse exponential function of porosity [17]. The high porosity of the CPSP cements originates from the low powder to liquid ratio (PLR = 2.0–3.0 g/ml) to form workable cement pastes ("plastic limit") rather than from incomplete cement setting. It would seem reasonable that further strength enhancement could be obtained by further reducing porosity, e.g. by ionic modification which acts to increase the zeta potential of the particles' surfaces thus increasing electrostatic repulsion. This can be effected by the addition of non toxic alpha-hydroxyacid salts to the liquid phase [18].

Due to the strong alkaline pH value of these cements, these materials should provide antimicrobial properties similar to $Ca(OH)_2$/salicylate cements [19] and CaO-Phosphate based cements [20] for the use as dental pulp capping agent, cavity liners or in the treatment of infected bone (osteomyelitis) where currently antibiotic doped PMMA-cements or -chains are used with the disadvantage of having to be removed after drug release and the risk of the formation of bacterial resistance if the released antibiotic doses are too low. In contrast, the cements reported in this study obtain their antimicrobial potential from the OH^- ions formed by the setting reaction of the cements (Equation (1)). The dissolution of the potassium/ sodium containing nanoapatite cement to calcium deficient hydroxyapatite according to Equation (3) would additionally lead to the release of strong basic sodium and potassium phosphates.

$$\begin{aligned} &9Ca_5K_2Na_2(HPO_4)(PO_4)_4(H_2O) \\ &\quad \rightarrow 5Ca_9(PO_4)_5(HPO4)(OH) + 9(K, Na)_2HPO_4 \\ &\qquad + 6(K, Na)_3PO_4 + 4H_2O \end{aligned} \tag{3}$$

5. Conclusion

High energy ball milling of crystalline $Ca_2(K,Na)(PO_4)_2$ in ethanol led to a mechanically induced phase transformation from the crystalline to

the amorphous state. This effect was responsible for an increase of the solubility of the material with grinding time and for a setting reaction to potassium substituted hydroxyapatite. Compressive strength of this cement system increased with prolonged grinding despite an increase in cement porosity. This paper demonstrated that mechanically induced changes in crystallinity represent a valuable processing route by which to produce novel low temperature inorganic biomaterials.

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