Modification of Calcium Phosphate Cement with α-Hydroxy Acids and Their Salts

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Calcium phosphate cements (CPCs) are usually modified by organic/inorganic additives to improve their mechanical performance and to adjust their rheological and setting properties to clinical requirements. In this work we used several nontoxic and biocompatible α-hydroxylated organic acids (glycolic, lactic, malic, tartaric, and citric acids) and their calcium and sodium salts for the modification of CPC. The unmodified cement used in this study consisted of an equimolar powder mixture of tetracalcium phosphate (TTCP) and dicalcium phosphate anhydrous (DCPA) mixed with water at a powder mass/liquid volume ratio of 3.3 g/mL. It had a compressive strength of 38 MPa and an initial setting time of 8 min. The free acids as cement liquids had mainly a detrimental effect on the strength of the cement and led to a decreased setting time around 2−4 min, while the calcium salts did not significantly alter the cement properties. However, the sodium salts of the oligocarboxylic acids (malic, tartaric, and citric acids) resulted in a liquefying effect combined with a strong reinforcement of the mechanical strength, such that compressive strength increased to 78−99 MPa. The liquefying effect and prolonged setting time of these compounds was thought to derive from a strong increase in the surface charge of both reactants and the reaction product hydroxyapatite as determined by ζ potential, which increased from about −15 and −18 mV in pure water for TTCP and DCPA, respectively, to values around −40 to −50 mV. In contrast, the calcium salts did not alter ζ potentials due to the formation of neutral and stable complexes in aqueous solution.

Introduction

The replacement of lost hard tissue, caused by trauma, disease, or congenital deformity requires the use of artificial materials to fill space and to prevent fibrous tissue in-growth during healing, to restore cosmetic appearance, and to act as scaffold for new bone formation. Synthetic materials offer the advantages of ease of procurement, having well-defined architecture and reproducible composition compared to autologous bone grafts; materials currently used include porous blocks of hydroxyapatite (HA),[1] granules of resorbable β-tricalcium phosphate with controlled morphology,[2] cements based on polymeric poly(methyl methacrylate) (PMMA),[3] bioglasses,[4] degradable polyesters,[5] or calcium phosphate cements.[6] The latter combine the advantage of being freely moldable and adaptable to bone defects with the excellent biocompatibility of calcium phosphate compounds and the benefit of low-temperature formation such that organic molecules and even living cells[7] may be incorporated into the cement. Brown and Chow[8] first reported the formation of an apatitic cement consisting of a mixture of tetracalcium phosphate (TTCP) and dicalcium phosphate anhydrous (DCPA); other formulations consisting of an α-tricalcium phosphate (TCP) combined with DCPA, dicalcium phosphate dihydrate (DCPD), or calcium carbonate have been described. Cement formation is often based on the different pH-dependent solubilities of calcium phosphates with respect to either HA or DCPD.[9] In contrast to PMMA-based cement,[10] the setting reaction of CPC occurs with minimal exotherm at a physiological pH value without the release of monomer. Several apatitic cements are approved for repair of cranial defects in humans.[11−13] However, the use of apatitic cements is limited to non- or low-load-bearing applications due to their poor mechanical performance relative to bone.[14] Kingery[15] is believed to have been the

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Table 1. Influence of Various Additives on the Basic Properties of Calcium Phosphate Cements (MCPM, Monocalcium Phosphate Monohydrate)

cement composition	additive	properties	ref
TTCP/DCPA	sodium phosphates	effective setting accelerator widely used in cement formulations	16
α-TCP + 2% HA seeds	in situ polymerization system of acrylamide/ammonium polyacrylate	formation of an ionically cross-linked material	17
MCPM/CaO/ DCPD/$Ca(OH)_2$	phosphorylated chitosan	increase of compressive strength, prolonged setting time	18
β-TCP/H_3PO_4	sulfate, pyrophosphate, and citrate ions	setting retardants for brushite forming cements	19, 20
α-TCP TTCP/DCPA	citric, acetic, and lactic acids	setting retardants, slight improvement of injectability and strength	21−24
α-TCP	gelatin	increase of compressive strength and formation of pores with 20−100 μm diameter	25
β-TCP/HA	hydroxypropylmethyl cellulose	improved injectability; prevents filter-pressing by increase of viscosity	26
TTCP/DCPA	mannitol crystals	formation of macropores after leaching out the crystals	27
α-TCP	sodium dodecyl phosphate	formation of macropores by air-entraining	28
TTCP/DCPA	sodium citrate	liquifier	29
α-TCP + 2% HA	various organic and inorganic additives, e.g. agar, gelatin, casein, alginic acid, sodium dodecyl sulfate, and glutardialdehyde	inhibition of setting with carbonate, pyrophosphate, and magnesium ions; considerable retarding of setting reaction with organic additives	30

first to report the formation of calcium phosphate cements as part of a larger study of metal ion phosphate cements. He proposed that acid−base systems produced the strongest cements when the rate of reaction was slow. Although largely unattributed to this work, this principle is commonly used to modify calcium phosphate cements, especially cements forming brushite that proceed via a fairly vigorous reaction between a basic calcium phosphate and phosphoric acid. In addition to modifying the rate of reaction, some additives are used to alter the rheology of the paste or to act as porogens. A summary of common modifications is shown in Table 1.

The strength of cements is highly dependent on their porosity; thus, reducing the amount of water used in mixing can increase strength. However, a limit is reached when there is inadequate water to form a workable paste, as weak and inhomogeneous cements are then formed. We have shown previously that citrate ions can reduce viscosity of apatite cements. This had three important consequences; high powder mass to liquid volume (M_p:V_l) ratio mixes were still workable and thus produced stronger cements, mixes could be compacted to produce very low porosity (∼15%), high strength materials,[29] and cement mixes were deliverable for the first time through hypodermic needles. This latter finding enabled both apatite and brushite calcium phosphate cement mixes to be delivered via hypodermic needles.[31,32]

Citric acid is an α-hydroxy triacid found in many plants and significantly in human bone mineral.[33] It is a chelating agent that has been used to inhibit CPC setting at low concentrations.[23] Relatively few nontoxic compounds are available with which to modify biomaterials; thus, our finding is highly significant. Within the class of α-hydroxy acids there are an assortment of nontoxic substances that may also have an effect similar to citrate. The aim of this study was to investigate the effect of lactate, glycolate, malate, tartrate, and citrate ions on the properties of apatitic CPC. These additives were used as solutions of sodium and calcium salts. Changes in mechanical and setting performance and modification of the cement setting reaction were compared and likely surface ion conformations modeled.

Experimental Section

The cement used for experiments was similar to that previously reported[29] based on the reaction between basic TTCP and acidic DCPA. TTCP was prepared by sintering an equimolar mixture of DCPA (Baker) and calcium carbonate (Baker) at 1500 °C for 18 h followed by quenching in air. The sintered cake was ground with pestle and mortar until it passed through a 355 μm sieve. The material was milled in agate jars to a particle size of 10−15 μm as determined by measurements of a suspension of powder in 2-propanol using a laser particle sizer Horiba LA-300 (Kyoto, Japan). DCPA was milled in 96% ethanol by means of a ball mill for 24 h. TTCP and DCPA were mixed at a ratio of 0.91 in a ball mill for 1 h. A 0.97 wt % amount of dry sodium phosphate accelerator (2:1 molar mixture of Na_2HPO_4 and NaH_2PO_4, both Merck, Germany) was mixed with the cement in order to accelerate the initial setting time of the cement mixture with water to 8 min at room temperature and 4−5 min at 37 °C.

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Cements were formed by mixing cement powder with an aqueous solution on a glass slab for 30 s with M_p:V_l = 3.3 g/mL. The liquid phase used was an aqueous solution of either the acid or sodium or calcium salt. All solutions were 500 mM except those with glycolic acid/calcium glycolate (100 mM) because they were less soluble; it was impossible to prepare calcium tartrate solution as it was insoluble. Control samples were made with water as the liquid phase.

Initial setting times of cement pastes were measured at room temperature using the Gilmore needle technique. Setting time was measured ($n = 3$) as the time between powder and liquid mixing and when the Gilmore needle ($d = 2.11$ mm, weight = 112 g) made no impression on the cement surface. Specimens for compressive testing were made by loading the cement paste into a stainless steel mold and biaxially compressing at a pressure of 2.7 MPa. Uncompacted cements were fabricated at M_p:V_l ratios between 4 and 5 g/mL using water and 500 mM sodium citrate, tartrate, and malate solutions. Cements were stored in 100% humidity at 37 °C for 24 h before testing. To determine ultimate compressive strength, specimens ($n = 12$) with a diameter of 6 mm and height of 12 mm were loaded under compression at a crosshead speed of 1 mm/min in a universal testing machine (Instron, 5544, Bucks, U.K.) until failure. Statistical significance was determined by one-way analysis of variance followed by the Tukey post hoc test, (SPSS v.10). Apparent density was found by dividing the mass of the dried specimen by the measured volume. The strut density was measured with helium pycnometry (Accupyc 1330, Micromeritics, Dunstable, U.K.) and relative porosity calculated from these measurements.

X-ray diffraction patterns of the set cements were recorded on a D5005 diffractometer (Siemens, Karlsruhe, Germany). Data were collected from $2\theta = 20-40°$ with a step size of 0.02°, and the count time was normalized to 1 s/step. The phase composition was checked by means of JCPDS reference patterns for TTCP (PDF ref no. 25-1137), HA (PDF ref no. 09-0432), and DCPA (PDF ref no. 09-0080). Medium crystal sizes of precipitated HA (based on the (321), (212), (300), and (202) peaks) and the crystal size in the [002] direction of HA as well as quantitative phase compositions of the materials were calculated by means of total Rietveld refinement analysis with the TOPAS software (Bruker AXS, Karlsruhe, Germany). As references, database structures of TTCP, HA, and DCPA were used together with a Chebychev fourth-order background model and a Cu Kα emission profile. FTIR spectra of set cements were recorded in transmission (Nicolet 320, Germany) of pressed KBr disks with a spectral resolution of 4 cm^{-1}. The effective surface charges of the TTCP, DCPA, and precipitated HA particles in contact with an aqueous electrolyte were determined from ζ potential measurement. Analyses were performed on a Zeta-Sizer 3000 (Malvern Instruments) in double distilled water and with various 50 mM solutions of electrolytes. Measurements were performed 10 times, and the average potential and the standard deviation were calculated.

In an attempt to correlate observed mechanical properties with liquid-phase additive molecular structure, energy minimization was carried out using the semiempirical quantum mechanics program MOPAC[34] with molecular geometry optimized using PM3 charges.[35] Multiple calculations were performed on all five pure α-hydroxylated acids and their sodium salts with each carboxylate group ionized and coordinated to a sodium ion. The surface structure could not be included in these energy calculations but was unlikely to have affected any of the resulting conformations except that of the citrate.[36] HA crystals have been shown to display a pronounced needlelike morphology with the fastest growth rate in the [001] direction.[37,38] Hence, the HA surface used in the calculation was based on cleavage of the bulk crystal structure[39] along the (101) plane, corresponding to the dominant exposed surface of HA crystals and such that the hydroxy groups are present along the surface structure. No calculations were attempted using TTCP or DCPA because a suitable surface structure or cleavage direction could not be identified in either material.

Table 2. Effect of Liquid-Phase Composition on Strength, Porosity, and Setting Time of Compacted (2.7 MPa) Apatite Cements[a]

liquid phase	UCS (MPa) ($n = 12$)	porosity (%)	setting time (min) ($n = 3$)
water	36.8 ± 4.5	33.6	8.17 ± 0.29
glycolic acid	27.7 ± 1.6	31.0	2.00 ± 0.00
sodium glycolate	44.6 ± 4.8	34.1	7.00 ± 1.00
calcium glycolate	26.6 ± 5.0	34.5	3.00 ± 0.00
lactic acid	24.8 ± 2.6	33.2	1.83 ± 0.29
sodium lactate	36.5 ± 6.3	31.4	6.00 ± 0.00
calcium lactate	33.1 ± 5.5	32.3	7.67 ± 0.58
malic acid	30.3 ± 5.1	29.4	2.00 ± 0.00
sodium malate	78.2 ± 5.3*	27.1	9.00 ± 1.00
calcium malate	55.1 ± 5.6	31.0	6.33 ± 0.29
tartaric acid	10.4 ± 1.2	35	1.00 ± 0.00
sodium tartrate	99.9 ± 10.1*	27.5	33.17 ± 1.44
citric acid	10.6 ± 1.7	30.0	6.0 ± 0.50
sodium citrate	82.5 ± 13.3*	24.6	42.0 ± 1.00
calcium citrate	39.2 ± 4.6	29.4	9.33 ± 0.29

[a] Strength values marked with asterisks (*) are significantly ($p < 0.001$) higher than others.

Results

Setting times, ultimate compressive strengths (UCS) and relative densities of the cements are given in Table 2. Using water as the liquid phase, the cement paste hardened in ∼8 min at room temperature. Setting times were strongly decreased to values of 3 min or less by using the free acids. Only slight variations (±2 min) in setting times were observed through the use of the calcium salts, and only calcium glycolate decreased the setting time significantly to 3 min. In contrast, trisodium citrate and disodium tartrate had a dramatic effect, prolonging the setting time to 42 and 33 min, respectively, while the other salts appeared to have little effect on setting time.

Compressive strengths of the cement mixed with all α-hydroxy acid solutions investigated as liquid were decreased when compared to water alone (e.g. values ranged between 10.6 MPa (citric acid) and 30.3 MPa (malic acid) and 36.8 MPa (water)). Compressive strengths found for calcium salts as liquid additives were mostly similar to the control with the exception of calcium malate with a mean compressive strength of ∼55 MPa. However, significant ($p < 0.001$) strength improvement of the cement matrix occurred when they were fabricated from mixes made with trisodium citrate (82.5 MPa), disodium malate (78.2 MPa), and disodium tartrate solutions (99.9 MPa), while neither the sodium glycolate nor lactate had any influence on compressive strength.

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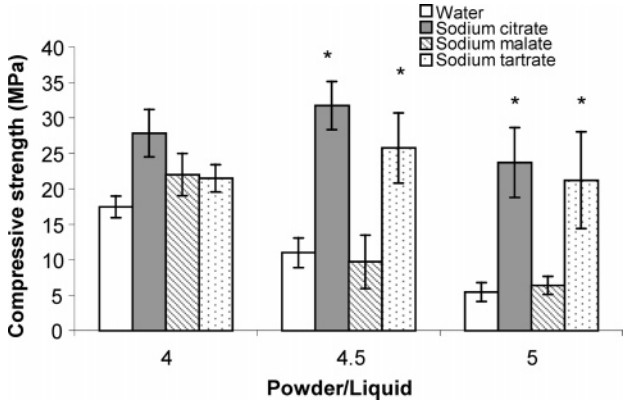

Figure 1. Effect of M_p:V_l ratio and liquid-phase composition on compressive strength of uncompacted apatite cements. Asterisks (*) mark values significantly higher than other values in the same group ($p < 0.001$).

Table 3. Effect of Liquid Phase Additives on Apatite Cement Phase Composition and Crystal Size of HA

liquid phase	% HA	% DCPA	% TTCP	av size HA (nm)	HA size from (002) peak (nm)
water	86	<1	14	15.7	19.8
glycolic acid	82	<1	18	13.4	43.3
sodium glycolate	76	<1	24	14.1	38.9
calcium glycolate	88	<1	12	16.1	38.6
lactic acid	88	<1	12	14.7	43.0
sodium lactate	89	<1	11	16.5	37.9
calcium lactate	79	<1	21	15.5	36.6
malic acid	88	<1	12	12.6	21.9
sodium malate	84	<1	16	13.3	50.0
calcium malate	82	<1	18	12.8	30.2
tartaric acid	79	<1	20	13.3	30.4
sodium tartrate	83	<2	15	10.6	22.6
citric acid	80	<2	18	13.1	35.8
sodium citrate	70	<1	30	12.4	33.4
calcium citrate	88	<1	12	17.1	39.4

The porosity of cements varied over a narrow range (35−25%) as might be expected for cements loaded to the same level of precompaction, with cements made from water and acid solutions generally exhibiting higher porosities than sodium salt solutions. Cement made with tartaric acid had the highest porosity and lowest strength, while that made with sodium tartrate had the lowest porosity and highest strength. Although strength improvements were quite marked, these systems were compacted. Another route to porosity reduction is to reduce the water content of the mix; however, if insufficient wetting is obtained, inhomogeneous setting results in a weak material. Figure 1 presents the effect of increasing M_p:V_l ratio of uncompacted cements made with water and the best additives identified from compaction experiments on compressive strength. As the M_p:V_l ratio increased, water and sodium malate containing cements decreased in strength from 17−22 to 5−6 MPa. However, maximal strength values were obtained at M_p:V_l = 4.5 g/mL for sodium citrate and tartrate containing cements (32 and 26 MPa, respectively).

The phase composition of the cements after setting for 24 h (according to Rietveld refinement analysis of the X-ray diffraction data) are summarized in Table 3. Phase analysis found that, for nearly all liquid components, the HA content after 24 h setting at 37 °C was typically between 80 and 90%, regardless of the kind of liquid phase. Only lower degrees of conversion (<80%) were obtained for sodium glycolate, calcium lactate, sodium citrate, and tartaric acid. The mean crystal size of the HA, calculated from the X-ray diffraction peak width by means of Rietveld refinement analysis, was typically 11−17 nm. While crystals were nearly isotropic for water as liquid phase, the crystal size determined from the (002) diffraction peak was approximately 2−3 times higher than the mean size for all organic additives.

ζ potentials of cement components TTCP, DCPA, and precipitated HA are displayed in Tables 4 and 5 for sodium and calcium salt solutions, respectively, as liquid phases. ζ potential measurements for the free acids were not possible because the acids dissolved the surfaces of the reactants forming calcium carboxylates. The particle surfaces of DCPA and TTCP were highly charged in sodium salt solutions due to the adsorption of ions with ζ potentials of about −36 to −50 mV compared to pure water with a ζ potential in the range between −15 and −18 mV. While the surface charge of HA in all salt solutions was higher than in water (−2 to −10 mV), in malate, tartrate, and citrate solutions it was double that (∼−40 mV) of particles in sodium lactate and glycolate solutions (∼−20 mV). In contrast, the calcium salts of the acids showed no significant increase on the surface charge of the cement particles.

To investigate whether carboxylate ions were incorporated into the cement product crystal structure, FTIR spectra of the modified cements were recorded before and after washing in water. Figure 2 presents the spectra of the cement modified with lactic acid, calcium lactate, and sodium lactate before and after washing the cement powder three times with water. Lactate ions were detectable in the cement after setting as indicated by the typical C−H vibrational bands around wavenumbers 2800−3000 cm^{-1}. These bands largely disappeared after washing the crushed cement powders, indicating that the ions were not significantly incorporated within the reaction product (whether added as acid or salts). Results were similar for the other α-hydroxy carboxylic acids and their salts, suggesting that the organic ions were mostly present as water-soluble salts, i.e., by the presence of weak metal carboxylate vibrations around 1400−1500 cm^{-1} and the absence of the strong carbonyl vibrations around 1700 cm^{-1} for carboxylic acid.

Discussion

Various additives for CPC are described in the literature (Table 1), mainly acting as setting accelerators (sodium

Table 4. ζ Potentials of Cement Reactants and HA Product in Sodium Salt Solutions

	ζ potential (mV) of particles in given solution					
compound	water	glycolate	lactate	malate	tartrate	citrate
DCPA	−18.4 ± 1.9	−38.6 ± 0.7	−40.3 ± 0.7	−42.2 ± 0.7	−41.7 ± 2.1	−50.1 ± 1.0
TTCP	−15.0 ± 1.8	−37.5 ± 1.2	−36.2 ± 2.0	−40.1 ± 0.7	−40.9 ± 2.2	−50.6 ± 3.8
HA	∼−2 to −10	−19.0 ± 1.0	−20.2 ± 0.6	−39.1 ± 0.6	−40.5 ± 1.4	−44.7 ± 2.4

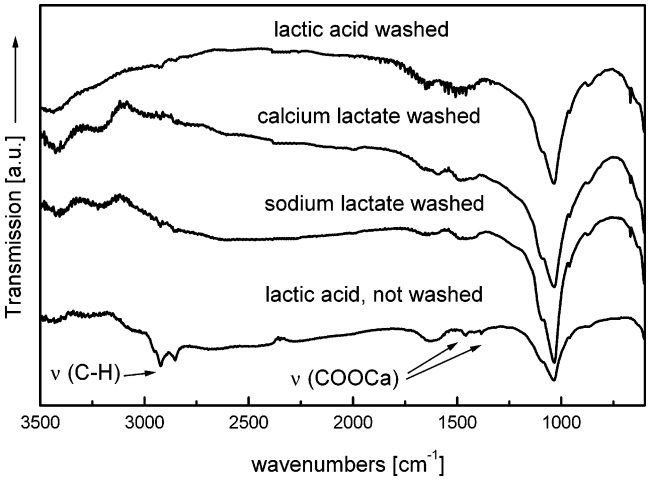

Figure 2. FTIR spectra of cements made with lactate ion containing liquid phases before and after washing in water.

Table 5. ζ Potentials of Cement Reactants and HA Product in Calcium Salt Solutions

	ζ potential (mV) of particles in given solution				
compound	water	glycolate	lactate	malate	citrate
DCPA	−18.4 ± 1.9	−1.1 ± 0.8	−11.2 ± 0.9	−10.6 ± 1.2	−18.7 ± 0.7
TTCP	−15.0 ± 1.8	−2.5 ± 1.2	−12.0 ± 1.5	−9.1 ± 0.8	−12.6 ± 2.7
HA	∼−2 to −10	−1.4 ± 1.3	−6.2 ± 1.3	−7.6 ± 0.7	−17.4 ± 0.4

phosphates) or retardants (citric acid, pyrophosphate, sulfate) or they improve the workability of the cement paste by greatly increasing the viscosity of the liquid phase (chitosan, cellulose derivates), making the paste puttylike and preventing wash-out when immersed in water prior to setting. Improvements in strength as a result of the additions are, however, limited to a few additives, e.g. phosphorylated chitosan[18] or a recently reported in situ polymerization system based on acrylamide/ammonium acrylate,[17] in the former case by porosity reduction and in the latter by the formation of an ionically cross-linked organic structure within the cement matrix.

In this work, the use of several sodium salts of α-hydroxylated mono-, di-, and triorganic acids enabled strength improvement by means of viscosity reduction of TTCP/DCPA cement mixes. All these salts are nontoxic and either are present in the body (citrate) and/or are orally nontoxic. Modification of the TTCP/DCPA cement matrix with α-hydroxy carboxylic acids resulted in a change of cement properties dependent on the number of carboxylic groups and the structure (acid, calcium/sodium salt) of the compounds. No improvements in mechanical performance or rheological properties were found by using the free acids. This was probably related to the strongly acidic character of the materials and the formation of a high initial calcium concentration by an acid−base reaction during initial setting, as indicated for the reaction of glycolic acid and DCPA in eq 1:

$$CaHPO_4 + CH_2OH{-}COOH \rightarrow Ca^{2+} + CH_2OH{-}COO^- + H_2PO_4^- \quad (1)$$

This strong increase in the initial Ca^{2+} concentration has been previously described for acetic acid and citric acid in a TTCP/DCPA cement by Tenhuisen and Brown;[23] the initial increase in Ca^{2+} concentration leads to a higher supersaturation of the cement liquid, which may explain the shorter setting times for the use of the acids due to more rapid HA precipitation.

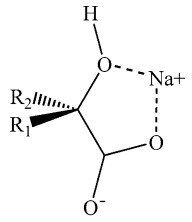

Figure 3. Five-membered ring binding the sodium ion to an α-hydroxy carboxylate group.

Changes in the rheological and mechanical behavior of the cement pastes made with sodium salts of di- and triacids were thought to occur as a result of modification of the solid/liquid interface of the cement reactants and product. One main factor that controls the viscosity and packing density of powder suspensions is the surface charge of the particles, which can be assessed from ζ potential measurements. A high value of surface charge improves the dispersion of fine particles in the micrometer to sub-micrometer range as a result of the mutual repulsion of particles in liquid phase.[40] In this study we showed that the ζ potential of the reactants could be altered by adding sodium salts of α-hydroxy acids to the liquid phase, but critically only di- and triacids increased the ζ potential of the product. The use of calcium salts did not significantly affect the potential at the solid/liquid interface of the cement particles. This may have been as a result of minimal interaction between the surfaces and the chelated α-hydroxy carboxylate ions.

In the case of the sodium salts there are discrete free hydroxy−carboxylate ions in the cement liquid at an almost neutral pH value prior to mixing. Here the high concentration of sodium ions within the liquid (0.5−1.5 M) decreased the stability of calcium−hydroxy carboxylic acids such that free ions remain present in the liquid and could adsorb on the particle surface increasing the surface charge. All sodium salts increased the ζ potential of the reactants TTCP and DCPA up to −40 to −50 mV. However, as determined qualitatively, liquefying effects were only obtained if the ζ potential of the reaction product HA was increased to values of <-35 mV when citrate, tartrate, and malate salts were used. The pronounced effect of sodium malate, tartrate, and citrate can be explained by the ability of the non-α-carboxyl group(s) to coordinate with excess sodium ions, thus preventing further interaction with other cement particles. This is clearly not possible in the pure acids or with the monovalent salts in which the α-hydroxy group is available for further coordination. In many metal chelates, the α-hydroxycarboxylate group of both malates and citrates is reported as being planar, forming a bidentate chelating group resulting in the formation of a five-membered ring binding the sodium ion (Figure 3).[36]

However, in this case, the absence of carbonyl bands in the FTIR spectra (Figure 2) implies that the sodium ion forms

(40) Gbureck, U.; Probst, J.; Thull, R. *J. Biomol. Eng.* **2002**, *19*, 51.

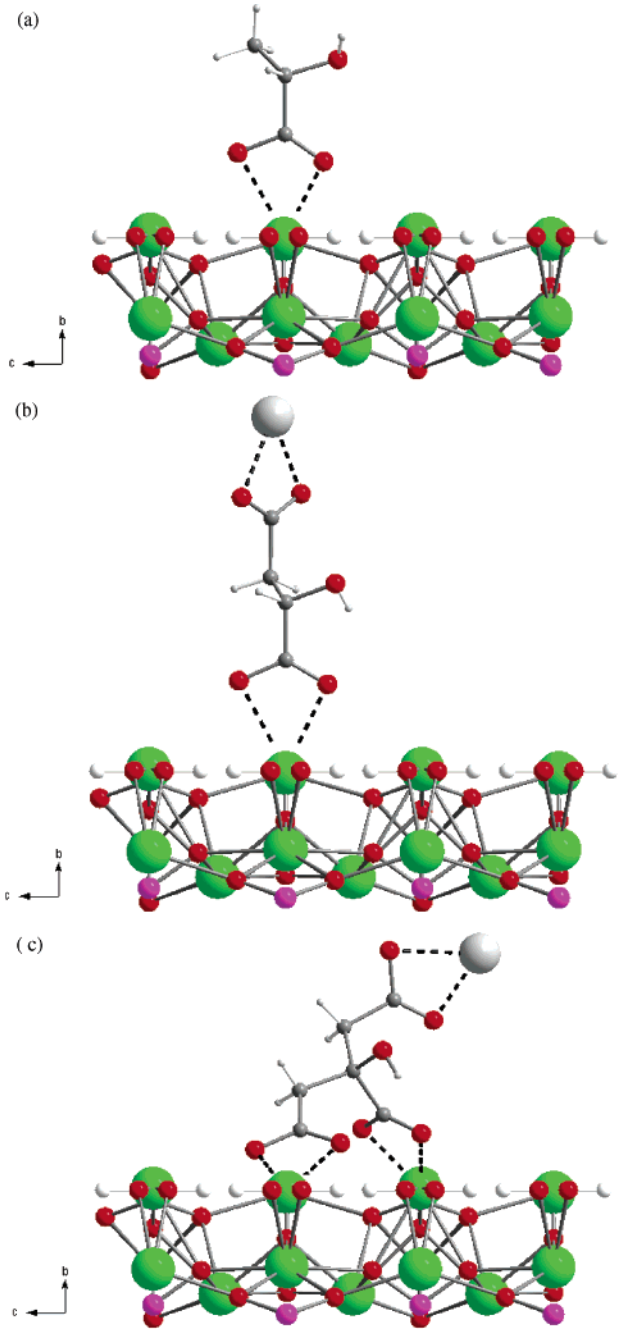

Figure 4. Possible conformations of sodium (a) lactate, (b) malate, and (c) citrate on HA surface. Ca···O and Na···O interactions are depicted by black dashed lines, and both atoms and ions are shown by solid spheres: Ca (green), P (purple), O (red), C (medium gray), Na (large light gray), and H (white).

a bidentate coordination with the other carboxylate group (or either of the terminal carboxyl groups in the case of the citrate) and that the α-carboxyl group is bound to a calcium on the HA surface with the α-hydroxy group sterically blocked. Indeed this was confirmed by energy minimization modeling. Parts a−c of Figure 4 illustrate the possible binding conformation of (a) lactate, (b) malate, and (c) citrate to the (101) HA surface through bidentate Ca···O interactions with other carboxylate groups capped by monovalent sodium ions via bidentate Na···O interactions.

It seems probable then that in the case of di- and triacids (Figure 4b,c) the surface effectively "seen" by other particles in the cement suspension is one of mutually repulsive bound sodium ions, while in the case of the monoacids the α hydroxy group is conformed in such a way that aggregative interparticulate bonding could occur. There are two possible ways in which the citrate can bind to the HA surface. The structural model presented here (Figure 4c) displays the citrate conformation in which only one of the terminal carboxyl groups coordinates to a sodium ion, with both of the remaining carboxyl groups bound to adjacent surface calcium atoms (Ca···Ca distance of 6.88 Å). Alternatively, the citrate could be bound through only one carboxylate to the HA surface, with both terminal groups involved in coordination with sodium ions. Despite this second conformation being sterically more favorable, the formation of a second calcium−carboxylate bidentate interaction is more likely to be energetically favored. Further support for this configuration may be found from estimates of sodium citrate concentration. Assuming spherical particles, an approximate surface area of 400 m^2 for 3.3 g of HA can be calculated from a particle size of 15 nm (Table 3). Maximum coverage of the HA surface by the citrate ion is approximately 1 molecule/(98 $Å^2$), based on steric restrictions (four out of every six calcium atoms to bind the citrate). Thus, 4.08×10^{20} molecules or 6.8×10^{-4} mol cover 400 m^2, which in 1 mL gives a concentration of 680 mM. An effective concentration for viscosity reduction will be lower than this value since dispersion would only be effective before the setting reaction has proceeded to completion. We have previously practically demonstrated that a limiting sodium citrate concentration is reached above which no further improvement in properties were observed.[29]

Further work involving detailed modeling of both structural models over an extensive surface area is needed to elucidate which of these is the true citrate-surface coordination. The reason neither DCPA nor TTCP showed an apparent specificity of ζ potential increase with sodium α-hydroxy acid salts was not determined, but α-hydroxyl group−particle surface bonding is thought to occur on these materials. Furthermore since the HA rapidly becomes the predominant phase after mixing with water and it also coats the reactant surfaces, the surface modification of HA is thought to be the significant factor determining cement mix properties.

Compressive strength of the compacted cements made with sodium malate, citrate, and tartrate solutions increased to about 78−99 MPa compared to 38 MPa for water as liquid. A chemical or microstructural reinforcement mechanism can be excluded according to the present results: X-ray diffraction analysis showed no evidence of a higher degree of conversion to HA, nor did the α-hydroxy acid ions seem to be incorporated into the crystal lattice (as shown by FTIR). The only apparent effect of the sodium salts was a slight reduction in crystal size of the precipitated HA, probably caused by a changed dissolution/precipitation behavior of the reactant and product phases. However, differences in HA crystal size are too small to explain the strong increase of strength, and it is more likely that strength improvement is related to a reduction of porosity by precompaction of the paste and ejection of cement liquid (Table 2), since the strongest cements had 6−9% less porosity than the other

formulations. However, a linear fit was not obtained between logarithm strength and porosity as might have been expected.[29] This is thought to have been as a result of incomplete or inhomogeneous setting due to too little water being present in some formulations. However, Figure 1 clearly showed the liquefying effect of particularly sodium citrate and tartrate ions, enabling very low water content cements to be formed with much higher strength than cements made with water. While surfactant and dispersant liquefaction of cements is well-known as a means for reducing water-induced porosity,[41,42] ionic modification of surfaces by nontoxic α-hydroxy acid ion monovalent salts has not previously been employed to improve low-temperature bioceramic properties.

Conclusion

The interaction between particle surface and additives to the liquid phase are significant in determining the rheological and setting properties of calcium phosphate cements as well as the mechanical performance of hardened cement matrix. The adsorption of multivalent ions results in an increase of the ζ potential of cement reactants and product, which is responsible for a macroscopically liquefying effect, such that less water is required to form a workable cement paste and higher cement strengths could be obtained. This strength improvement will be useful for an application of the cements in some load-bearing areas, for example in upper spine surgery (vertebroplasty), while the liquefying effect can lead to injectable cements for minimal-invasive application techniques. The results of this work are the basis for the adjustment of defined cement properties in various cement systems, e.g. apatitic cements based on α-TCP or brushite forming cement mixtures, and it can be concluded that the nature of the interface between cement particles and the liquid phase plays a major role in determining cement properties.

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(42) Gauthier, O.; Khairoun, I.; Bosco, J.; Obadia, L.; Bourges, X.; Rau, C.; Magne, D.; Bouler, J. M.; Aguado, E.; Daculsi, G.; Weiss, P. *J. Biomed. Mater. Res.* **2003**, *66A*, 47.

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