

Calcium and zinc ion release from polyalkenoate cements formed from zinc oxide/apatite mixtures

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Abstract Calcium and zinc ion release from hydroxyapatite-zinc oxide-poly(acrylic acid) (HAZnO-PAA) composite cements into deionised water was investigated as a function of HA content, PAA concentration, PAA molecular weight and maturation time. At any given maturation time, zinc ion release was constant until the HA content was at the maximum loading (60 wt%) resulting in the cement matrix breaking up, allowing exacerbated ion release. The calcium ion release increased with increased HA content in the composite until the maximum loading where the release drops off. Up to this point, the release of both ionic species was proportional to square root time for the initial 24 hour period, indicating that the release is diffusion controlled. In agreement with related data from conventional Glass Polyalkenoate Cements (GPCs), it is the concentration of the PAA, not the molecular weight, that influences ion release from these materials. However, unlike GPCs, the release of the active ions results in a pH rise in the deionised water, more conventionally seen with Bioglass® and related bioactive glasses. It is this pH rise, caused by the ion exchange of Zn^{2+} and Ca^{2+} for H^+ from the water, leaving an excess of OH^- , that should result in a favourable bioactive response both *in vitro* and *in-vivo*.

1. Introduction

Smith [1] developed the first zinc polyalkenoate cement (ZPC) by reacting modified zinc oxide (ZnO) powder with aqueous polyacrylic acid (PAA). The acid reacts with the basic ZnO to form a cross-linked metal polyacrylate salt containing residual ZnO particles. Subsequently, Kent and Wilson [2] went on to develop the GPCs, based on acid degradable fluoro-alumino-silicate glasses and PAA. ZPCs and GPCs are used in dentistry as both adhesives and restorative materials. Polyalkenoate cements set at body temperature without undergoing any polymerisation shrinkage and without significant evolution of heat [3].

During the last ten years there has been considerable interest in the development and use of polyalkenoate cements for medical applications [4, 5]. These cements are claimed to be bioactive in the bone environment [6] as a result of the release of calcium, phosphate and fluoride ions, as well as due to the formation of a silicious gel phase in a similar way to Bioglass [7]. However recent research [8] has shown that aluminium ions released from conventional GPCs results in defective bone mineralisation and as a consequence the ability of these cements to chemically bond to bone is lost.

It is possible to form polyalkenoate cements from HA-fluorapatite [9] and HA-ZnO mixtures [10]. These cements would be attractive for medical use as preset bone substitutes and as *in situ* setting bone cements. Because such cements contain no aluminium, but have the potential to be bioactive (via the release of calcium, phosphate and fluoride or zinc ions), they should integrate well with bone tissue and retain the capacity for direct chemical bonding to bone.

Zinc is the second most prevalent trace element in the body and is required for proper cellular and immune function. It is essential in the synthesis of proteins and insulin [11]. The

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body needs zinc to metabolize carbohydrates, fats, proteins, and alcohol and to dispose of carbon dioxide. Zinc also assists in wound healing [12]. For example the prevalence of leg ulcers in the ageing population is related to a deficiency of zinc [13]. The zinc ion (Zn^{2+}) released from zinc-based cements enhances osteoconductivity [14]. The novel cements produced in this work are based on HA/ZnO/PAA mixes and as such contain a zinc-rich phase. Previous studies had determined the optimum composition of these cements in terms of rheology, mechanical properties and *in-vitro* behaviour [15–17]. The working and setting times of these cements are similar to conventional GPCs [15] and are not significantly affected until the HA content is present in quantities greater than 45 wt%. Such materials are an improvement on acrylic based cements due to their non-exothermic setting, improved chemical adhesion and because of their potential to release measured amounts of zinc to facilitate wound healing. The objective of this research was to determine the extent of both zinc and calcium ion release from these optimised materials, with respect to variations in HA content, PAA molecular weight and PAA concentration, in order to predict the likely response of using such composite cements *in vivo*.

2. Experimental

2.1. Materials

Medical grade HA was obtained from Stryker Howmedica Osteonics (Limerick, Ireland) and had a mean particle size of $24.5\mu\text{m}$ [18]. ZnO was supplied by Advanced Healthcare Limited (Tonbridge, UK) with a mean particle size of $17.4\mu\text{m}$ [18]. Two PAAs, E9 and E11 were obtained from CIBA speciality polymers (Bradford, UK). The molar mass details of the acids are supplied in Table 1.

2.2. Specimen fabrication

Cement samples were formed by first hand mixing the HA and ZnO at various ratios to produce a series of powders containing 0, 15, 30, 45 and 60 wt% HA; the HA content increasing at the cost of the ZnO. Cements were formed by mixing the powders with the PAAs (weight ratio, 3:1) and then adding 10% m/m (+) tartaric acid solution (weight ratio, 3.25:1). The acid content of the mixture being 50%. Specimens based on E9 acid with a concentration of 60 wt%

were also prepared, where the weight ratio of HA + ZnO + PAA:H₂O was 4.3:1.

2.3. Evaluation of ion release

Inductively Coupled Plasma Spectroscopy (ICP-S) was employed to evaluate the extent of Zn^{2+} and Ca^{2+} release from the cements. Cements were allowed to set in the appropriate mould ($8\text{ mm}\varnothing \times 2\text{ mm}$ thickness) for one hour ($37 \pm 2^\circ\text{C}$), then demoulded and placed in a 20 ml aliquot of deionised water (37°C). The aliquots were changed at intervals of 30 mins, 1 Hr, 2 Hrs, 4 Hrs, 8 Hrs, 12 Hrs, 18 Hrs, 1, 7 and 28 days. Following the removal of the specimens, the ion concentration in the liquid was measured on a Perkin-Elmer Optima 3200 XL (Norwalk, CT 06859, USA). Glassware was washed in concentrated hydrochloric acid (HCl) and rinsed with deionised water. All the analyses were performed in triplicate and results were normalised against a sample of deionised water from the same source. The cumulative release per unit surface area (ppm cm^{-2}) against the square root of time ($\text{hours}^{1/2}$) was plotted. The pH of the water was also measured at the same time intervals using a pH meter.

3. Results and discussion

Cements were produced as outlined in the methods section. The cements were based on E9 (50% and 60% concentration) and E11 acids (50% concentration) and contained between 0 and 60 wt% HA. The effect of increasing HA (and conversely decreasing ZnO) content and maturation time on the calcium and zinc ion release was evaluated.

3.1. Influence of HA content on ion release

Tables 2 and 3 exhibit the zinc and calcium ion release from the E11/50 cement series, with respect to time. Standard deviations are available for the zinc release only.

Considering the recorded standard deviations, the zinc ion release of the cements appears constant, regardless of HA content, up to and including 45 wt% HA (Table 2). Consideration of likely experimental error suggests that small deviations in measuring out the reagents during the cement production could result in inaccuracies of up to 1 ppm. However, when HA content is increased to 60 wt.%, the cements demonstrate a significant increase in Zn^{2+} release presumably because the HA is starting to cause degradation in the cement system. The Ca^{2+} release increases with increasing HA content (Table 3). However, the highest HA containing materials (60 wt%) show a reduced Ca^{2+} release rate, opposed to the increased Zn^{2+} release for the same cements. The reason for this is unclear but may be related to the development of a more cross-linked cement matrix, thereby resulting in lower diffusion and a reduced ion release. Ca

Table 1 Molar mass details of the PAAs

Code	M_w	M_n	PD	Peak mol. wt.
E9	80,800	26,100	3.1	83,500
E11	210,000	64,400	3.2	186,000

Table 2 Cumulative zinc ion release (with standard deviations) per unit surface area (ppm cm^{-2}) of the cement series

Time (hours)	HA content (wt.%)				
	0	15	30	45	60
0.5	0.57 (0.26)	0.48 (0.09)	0.72 (0.28)	0.36 (0.04)	1.33 (0.07)
1	0.69 (0.22)	0.62 (0.06)	0.93 (0.31)	0.42 (0.04)	2.24 (0.38)
2	0.77 (0.21)	0.70 (0.08)	1.02 (0.52)	0.54 (0.07)	3.00 (0.41)
4	0.86 (0.21)	0.81 (0.13)	1.13 (0.54)	0.61 (0.07)	3.91 (0.78)
8	1.09 (0.21)	0.92 (0.14)	1.30 (0.52)	0.68 (0.08)	4.81 (0.91)
12	1.21 (0.18)	1.03 (0.15)	1.41 (0.50)	0.80 (0.16)	5.45 (0.99)
18	1.67 (0.16)	1.54 (0.24)	1.76 (0.55)	1.22 (0.23)	6.74 (0.99)
24	1.80 (0.15)	1.64 (0.27)	1.87 (0.53)	1.42 (0.16)	7.97 (0.96)
168	2.00 (0.15)	1.91 (0.31)	2.15 (0.56)	1.66 (0.13)	9.20 (1.36)
672	2.29 (0.22)	2.11 (0.30)	2.43 (0.51)	1.88 (0.18)	10.49 (0.36)

Table 3 Cumulative calcium ion release per unit surface area (ppm cm^{-2}) of the cement series

Time (hours)	HA content (wt.%)				
	0	15	30	45	60
0.5	0.00	0.59	15.44	14.56	11.0
1	0.00	0.84	30.44	30.00	23.0
2	0.00	1.61	31.46	44.81	23.6
4	0.00	1.86	31.8	44.96	24.1
8	0.00	2.69	31.86	45.07	24.6
12	0.00	3.03	32.03	45.22	25.1
18	0.00	3.31	32.48	45.78	25.4
24	0.00	3.42	33.36	45.85	25.9
168	0.00	3.46	33.40	45.92	27.9
672	0.00	3.90	33.50	46.09	29.1

ions released by the HA may both exchange with Zn ions in the crosslinked structure and enter the solution. Calcium and zinc ions have ionic radii of 0.099 nm 0.074 nm, respectively. As the ions are of different sizes this may change the strength of the cross-linking. Therefore ion release may not directly relate to the degradation or strengthening of the network.

3.2. Influence of maturation time on ion release

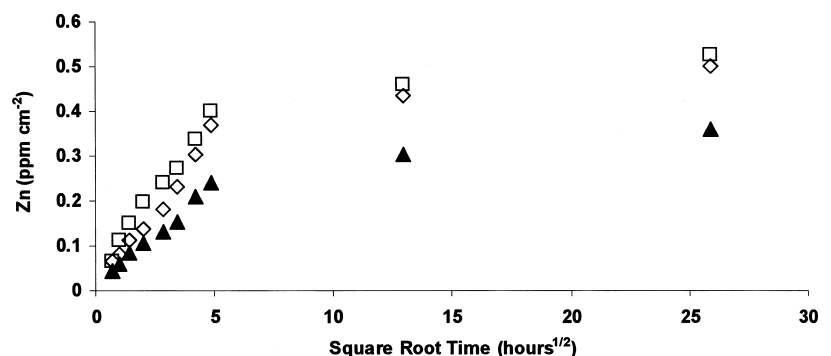
The initial zinc ion release up to one day from the E11/50% PAA concentration series was found to be proportional

to square root time (Figure 1). This also occurs with the lower molecular weight PAA (E9) at 50 and 60% concentration, suggesting that ion release is a diffusion controlled process.

The zinc ion release is not affected by PAA molecular weight. From Figure 1, the Zn²⁺ release from the E9/50% and E11/50% cements (both containing 50 wt% HA) are virtually identical. This correlates with previous work on both these cement systems [15] and on conventional GICs [19] which shows that PAA molar mass has no influence on setting chemistry or Young’s modulus. However, Figure 1 shows that PAA concentration may have some influence ion release. Going from 50% to 60% acid concentration appears to reduce the Zn²⁺ release, probably as a result of a more cross-linked cement matrix giving a lower zinc ion diffusion coefficient. However, although the reduction was evident at every duration the real changes are small (less than 1 ppm cm^{-2}) and repeat studies would be needed to confirm the influence of PAA concentration on ion release. As the ion release has been shown to be diffusion dependent, this lower diffusion coefficient would result in less zinc ion release.

The calcium ion release is also seen to be diffusion dependent. Evaluating the cumulative Ca²⁺ release with respect to HA content for the E11/50 cement series (Figure 2) it is evident that release stabilises after 2 hours of sample immersion in the distilled water.

Fig. 1 Cumulative Zn²⁺ release from the \diamond E9/50%, \blacktriangle E9/60% and \square E11/50% cement series with an HA content of 60 wt.%



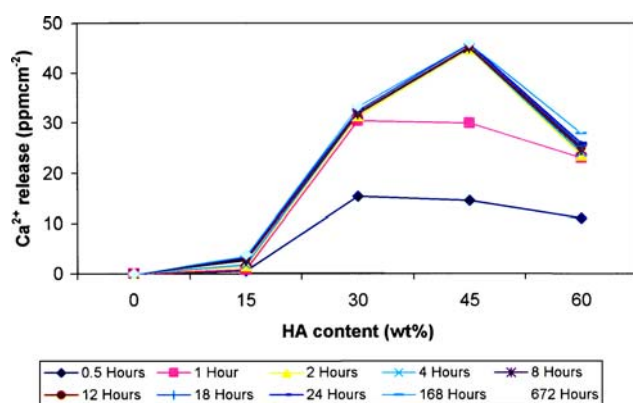
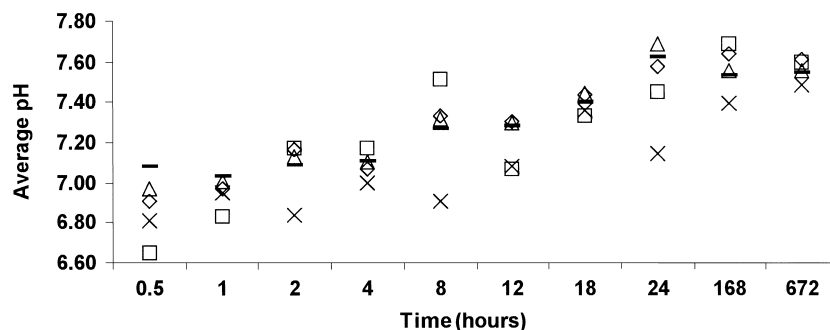


Fig. 2 Cumulative Ca^{2+} release with respect to the HA content of cements in the E11/50% PAA series

Fig. 3 Average pH of the E11/50 wt% cements including \square , \diamond , \triangle , ∇ and \times 0, 15, 30, 45 and 60 wt.% HA compositions.



3.3. Variation in pH of the deionised water

The cements exhibit a steady increase in pH with respect to time. The pH values of most of the compositions are similar for equivalent time periods. For example, after 18 hours, pH values of the E11/50 wt% cements are 7.3, 7.4, 7.5, 7.4 and 7.4 for 0, 15, 30, 45 and 60 wt% HA cements respectively (Figure 3).

There is a significant increase in pH of the medium with respect to time (Figure 3). This is due to the ion exchange of Zn^{2+} and Ca^{2+} ions for H^+ ions from the water, which will leave an excess of OH^- ions. This is in contrast to the fall in pH seen with conventional GPCs [20], which release fluoride ions (F^-) principally by an ion exchange process with OH^- . A pH rise similar to that exhibited here is seen with Bioglass® [7] and related bioactive glasses [21]. It is this pH rise that facilitates apatite deposition on the surface of Bioglass® when it is immersed in simulated body fluid (SBF) [7]. Kamitakahara et al. [22] claimed that GPCs are not bioactive as a result of the release of low molecular weight PAA chains dissolving from the cement giving rise to a low pH which inhibits apatite formation in SBF. However, they studied a commercial GPC based on a fluoro-alumino-silicate glass and the failure of this cement to form an apatite-like layer in SBF is more likely a result of fluoride ion release by an ion exchange process resulting in a decreased pH, rather than due to the release of low molecular weight PAA chains.

Low levels of zinc release are beneficial. Yamaguchi et al. [23] found that Zn^{2+} increases bone protein, calcium content, and alkaline phosphatase activity in rat calvaria at concentrations between 10^{-7}M to 10^{-4}M zinc in 2 ml of tissue culture medium. Ovesen et al. [24] examined the positive effects of zinc on skeletal strength in growing rats. They found that supplemental zinc increases bone size, bone mass, and strength in growing rats, indicating that it is a potent factor in bone metabolism. Indeed, recent animal trials reported in the literature [25] showed that levels of zinc release in the body exceeding those reported here (up to 10 mg of zinc per 100 mg body weight per day) resulted in increased bone mass with no reported side effects.

4. Conclusions

In agreement with related data from conventional GPCs, the concentration of the PAA, not the molecular weight, influences ion release from these novel materials. However, unlike GPCs, the release of the active ions results in a pH rise in the deionised water, more conventionally seen with Bioglass® and related bioactive glasses. It is this pH rise, most likely caused by the ion exchange of Zn^{2+} and Ca^{2+} for H^+ from the water, leaving an excess of OH^- , that should result in a favourable bioactive response both *in vitro* and *in-vivo*.

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