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Effect of molecular weight and concentration of poly(acrylic acid) on the formation of a polymeric calcium phosphate cement

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Previous investigations have noted that the tetracalcium phosphate (TTCP)/dicalcium phosphate anhydrous (DCPA) apatite forming calcium phosphate cement (CPC) possesses many favorable properties from a biomaterials standpoint. Despite these positive properties various shortcomings have limited clinical usage of these materials and fostered investigations into the effect of numerous additives. The present study concerns the effect of poly(acrylic acid) (PAA) addition and the influence of factors such as molecular weight and concentration of the additive on the properties of the set cement. One-way ANOVA was conducted using all results obtained, to firstly derive the influence of concentration within each molecular weight group, and secondly to derive the influence of molecular weight within each concentration group. All investigated mechanical properties were influenced by both molecular weight and concentration of the additive. Higher molecular weights tended to result in cements with shorter setting times and higher compressive, diametral and biaxial flexural strengths than their lower molecular weight counterparts. The effect of concentration on the properties of the set cement however was somewhat more complex, a negative correlation was observed between the initial setting time and PAA concentration. In regards to the final setting time, any correlation with concentration was difficult to derive as a consequence of the highly brittle nature of cements made with low concentrations. In regard to mechanical properties, intermediate concentrations tended to give higher strengths than both their higher and lower counterparts, however the exact pattern was largely specific to the mechanical strength test employed. We conclude that molecular weight and concentration of PAA influence the setting behavior and final mechanical properties of the TTCP/DCPA cement, and that selection of an appropriate PAA solution can lead to the production of cements with properties superior to those formed in the absence of the polymer.

Introduction

The tetracalcium phosphate (TTCP)/dicalcium phosphate anhydrous (DCPA) based calcium phosphate cement (CPC) is a self-setting apatitic cement developed by Brown and Chow [1]. The constituent precursors namely TTCP and dicalcium phosphate dihydrate (DCPD) or DCPA set to produce hydroxyapatite (HA) when mixed at an equimolar ratio in water. The setting reaction involves precursor dissolution that results in supersaturation of the cement liquid with respect to calcium and phosphate ions. These ions then combine to precipitate HA from solution as shown in Fig. 1. As a consequence of the nature of this reaction, the resultant HA possesses both crystalline and amorphous phases. This coupled with the ubiquity of this mineral in vertebrate hard tissues, results in a material that is both biocompatible and bioresorbable. In addition, the material sets at physiological pH and can be molded *in situ* to allow intimate association with the implant site. Hence, a great deal of attention has been paid to CPCs and they have been proposed and indeed used for a number of clinical applications [2–6].

Despite the inherent positive properties of CPCs such as the TTCP/DCPA based system, they tend to exhibit

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$$2Ca_4(PO_4)_2O + 2CaHPO_4 \rightarrow Ca_{10}(PO_4)_6(OH)_2$$

Figure 1 Setting reaction of TTCP and DCPA in the WSCPC.

relatively low mechanical strengths [1, 7] slow initial and final setting times [8], inadequate handling properties and solubility in aqueous solutions during the early stages of the setting reaction [9, 10]. The clinical usefulness of the cements is thus limited, especially in application to areas containing blood, tissue fluid and saliva [11–14] or in locations where load bearing is a requirement. These limitations have fostered investigations into the effects of several additives on cement properties, e.g. sodium hydrogen phosphate solution, sodium alginate [11, 12, 15]; hydroxypropyl methylcellulose, carboxymethly cellulose, chitosan lactate and chitosan acetate [9]; polymethyl vinyl ether-maleic acid [16, 17], glycerol dimethacrylate, pyromellitic dianhydride and other resins [10]; gelatin, polyvinyl alcohol, poly(acrylic acid) (PAA) and other water soluble polymers [18] lactic acid, glycerol, and chitosan [19].

A PAA additive was used in the present study with the aim of improving the properties of the water-setting CPC (WSCPC) to form a polymeric CPC (PCPC). The attempted improvements comprise the lowering of the solubility of the cement paste in aqueous media, a reduction in setting times and an increase in mechanical strength. In addition, the influence of molecular weight and concentration of the PAA on these properties were studied in detail. It is important to note that the setting reaction in the presence of the polymer is distinct from that which occurs in the WSCPC. The presence of PAA in a CPC results in a cement setting reaction that is more akin to a glass-ionomer or zinc polycarboxylate cement [16, 17]. The initial setting of glass-ionomer cements is associated with the formation of calcium poly(acrylate), with further, more complex maturation occurring in the longer term. Thus, the inclusion of PAA to the CPC is expected to firstly influence early solubility via the rapid formation of the insoluble calcium poly(acrylate) and secondly, the presence of cross-linked polymer chains within the cement structure is expected to enhance mechanical properties.

Materials and methods

Cement powder (for water setting CPCs)

TTCP was synthesized by reacting equimolar amounts of calcium carbonate ($CaCO_3$) and calcium hydrogen orthophosphate anhydrous ($CaHPO_4$) (DCPA) at a temperature of 1450 °C for 4 h [21]. X-ray diffraction was then conducted to ensure that the product was a pure phase TTCP. The DCPA precursor was obtained commercially (Fisher). Both precursors were milled separately in a 96% alcohol medium for 48 h in alumina reinforced porcelain jars with zirconia grinding media. Once the powders had been comminuted, grinding media were removed, and the alcohol evaporated at 80 °C prior to placement in a vacuum desiccator. Particle size analysis showed that both comminuted precursors possessed diameters in the region of 5 μm.

Water setting CPCs

Optimized water setting cements as described in the literature contain an equimolar mixture of DCPA particles ($\sim 1\,\mu m$) and TTCP particles ($\sim 11\,\mu m$) [7]. Comparable water setting CPCs cements were prepared and characterized under similar conditions to those of the experimental polymeric CPCs developed in this study, in order to assess the effect of the inclusion of PAA and the influence of its molecular weight and concentration. Although the particle sizes used to make the WSCPC in this and other studies [1, 7] differ from the optimized particle sizes used to make the PAA containing CPCs (PCPCs); this system was prepared to establish a standard WSCPC with well defined properties against which the properties of a cement containing a polymeric acid (PCPC) with an identical *P*/*L* ratio could be compared.

Cement powder (for PCPCs with PAA additive)

Pilot studies prior to the main investigation showed that usage of the fine TTCP particles ($\sim 5\,\mu m$) made by milling, led to an extremely fast rate of reaction, which meant that the production of a paste with uniform consistency was at best very difficult as setting was almost instantaneous. Other studies have observed that the increase in surface area as a result of TTCP comminution greatly increases the rate of reaction. As a consequence larger TTCP particles were used in the formulation of the polymeric cement powder**.** Investigations showed that TTCP particles between 45 and 75 μm in size were sufficiently large to allow the cement paste to be mixed to a uniform consistency prior to packing. Due to the low solubility of DCPA, this precursor could be used in comminuted form, and in addition smaller particles of at least one of the precursors is likely to result in a stronger cement structure. However for the production of the water-setting cements made for comparison, both precursors were used in their comminuted forms. Despite differing precursor particle sizes, for the production of both the WSCPC and CPC, equimolar amounts of the two precursors comprised the powder phase of the cement.

Powder to liquid ratio

Early experiments for production of the water-setting cement were conducted using a powder to liquid ratio (*P*/*L*) of 4 as reported in previous publications [1, 8]. However in this series of experiments it was observed that this *P*/*L* ratio was insufficient for the formation of a cement paste, the explanation for this observation is not clear but may relate to increased porosity in either precursor. Further experiments were then conducted to find a suitable *P*/*L* ratio. Ratios of 2, 2.5 and 3 were investigated. A *P*/*L* ratio of 3 did not give a cement paste with good consistency, and when allowed to set the resultant cement was found to be very weak. A *P*/*L* ratio of 2 resulted in an exceptionally slow setting cement with low compressive strength. However, when the *P*/*L* ratio was adjusted to 2.5, a HA forming, water-setting TTCP/DCPA based cement, with good consistency and a setting time of less than 4 h was produced, as a consequence a P/L ratio of 2.5 was selected for the formulation of both the water setting and polymeric cements tested in this study. The compressive strength of these water-setting cements were found to be comparable to those for other TTCP/DCPA based cements reported in the literature [1, 7] and a subsequent tukey one-way analysis of variance (ANOVA) comparison showed the absence of any significant difference ($p = 0.544$).

Initial and final setting times

Cements were prepared by mixing powder and liquid components in a porcelain pestle and mortar using a liquid/powder ratio of 2.5. The cement pastes were mixed for 20–30 s and the working and setting times were determined at room temperature and humidity using the standard Gillmore needle test (American National Standards Institute/American Dental Association Specification No. 61 for zinc polycarboxylate cement. *J. Am. Dent. A.* **101** (1980) 669–671). The light needle used had an applied weight of 115.12 g and a diameter of 2.13 mm, and the heavy needle a diameter of 1.02 mm and an applied weight of 453.6 g. Initial setting time is defined as the time elapsed, necessary for the light needle to no longer leave any visible deformation on the cement surface, i.e. is able to support a static pressure of 0.3 MPa and the final setting time is reached when the paste is able to support a static pressure of 5 MPa.

The effect of molecular weight variation on the properties of the cements was determined by using three different grades of PAA (Ciba Chemicals) as shown in Table I. Furthermore, the effect of concentration of the PAA additive on cement properties was studied by adding the different molecular weight PAAs to the cement powders at concentrations of 25%, 20%, 15% and 10% w/v, obtained via dilution of the stock solutions with distilled water where necessary.

Mechanical testing

Compressive and diametral tensile strength specimens were prepared by mixing the cement as aforementioned, and packing the cement paste into stainless steel molds pre-treated with a silicone releasing agent with inner dimensions corresponding to a cylinder with approximate length of 12 mm and a diameter of 6 mm.

Disc shaped specimens with a diameter of 8.5 mm and a thickness of 1.15 mm were prepared for the biaxial flexural strength (BFS) measurement. All specimens within molds were secured in G clamps after packing and placed in an incubator at a temperature of 37 °C during setting. Once set the cement specimens were removed and then placed in glass vials and immersed in distilled water for 16 h prior to mechanical testing. All mechanical tests were conducted using a calibrated Instron 1193 universal testing machine with a crosshead speed of 1 mm/min.

TABLE I Properties of PAA

Polyacid	Molecular weight
Glascol E9 (G-E9)	100 000
Glascol E7 (G-E7)	30 000
Dispex R50 (D-R50)	< 30 000

Statistical analysis

All results obtained were analyzed using Tukey one-way ANOVA to derive the statistical significance of the influence of concentration and molecular weight.

Results

The influence of molecular weight of PAA on cement

Initial and final setting times

Initial and final setting times (Figs. 2 and 3) appear to be greatly influenced by the molecular weight of the PAA used, the largest differences are between the two relatively high molecular weight PAA solutions, namely G-E9 and G-E7 and the low molecular weight PAA, D-R50, with the two higher molecular weight PAAs possessing much shorter initial and final setting times (T_i and T_f). A smaller but equally obvious effect is notable between G-E9 and G-E7 containing cements, with the G-E9 cements possessing shorter T_i and T_f than those containing the G-E7. It thus seems likely that within the range investigated that there is a negative correlation between molecular weight and initial and final setting times.

Mechanical properties

All mechanical tests conducted were influenced by the molecular weight of the PAA additive. The compressive strengths of the cements are shown in Fig. 4 and two

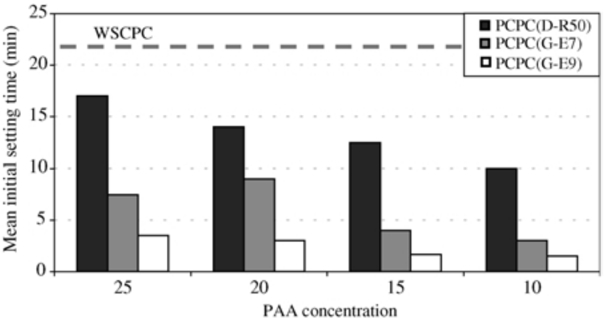

Figure 2 Working time (T_i) of polymeric cements.

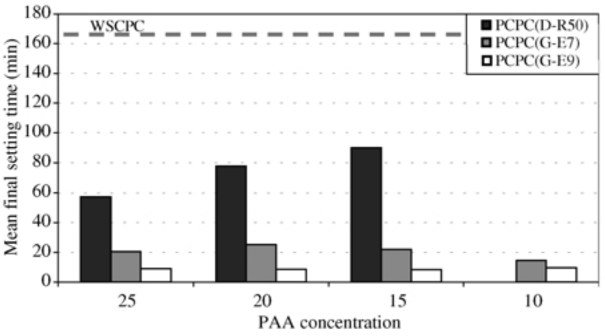

Figure 3 Setting time of polymeric cements.

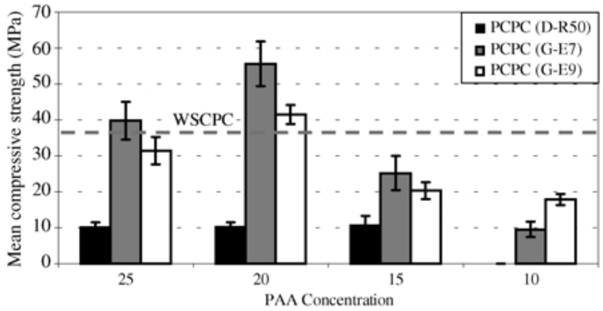

Figure 4 Compressive strength of polymeric cements.

PCPC formulations (PCPC with 20% G-E7 & PCPC with 20% G-E9) exhibit considerably higher compressive strengths (∼ 56 and 42 MPa, respectively) relative to the WSCPC produced as part of this study and those produced by other investigators (∼ 36MPa) [1, 7]. However, only the 20% G-E7 containing cement possesses a statistically significant ($p = 0.002$) improved compressive strength (Fig. 4) relative to the WSCPC produced as part of this study, resulting in a cement that is almost 20 MPa stronger. However, low concentrations (15% and 10%) of the high molecular weight PAAs (G-E9 and G-E7) led to cements that were significantly weaker than the WSCPC. In regard to the D-R50 containing cements it is apparent from the compressive strength results (Fig. 4) and has been confirmed by ANOVA that all cements containing the D-R50 were significantly weaker than both the WSCPCs made as part of this study and those quoted in the literature.

The polyacids G-E7 and G-E9 with the higher molecular weights generally yielded stronger cements than those containing D-R50. ANOVA of compressive strength and DTS results showed that at any given concentration, (with the exception of 15% in regard to compressive strength) that the G-E7 and G-E9 are significantly stronger than their D-R50 containing counterparts. In addition, the DTS results (Fig. 5) suggest that even amongst the higher molecular weight PAAs, i.e. G-E7 and G-E9, that higher molecular weights lead to cements with superior properties relative to their lower molecular weight counterparts, this observation was confirmed by ANOVA which showed that at any given concentration with the exception of 10%, that G-E9 cements were significantly stronger than G-E7 cements.

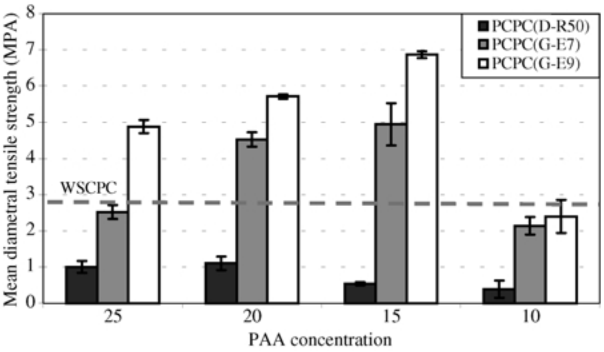

Figure 5 Diametral tensile strength of polymeric cements.

The influence of concentration of PAA on cement

Initial and final setting times

Concentration appears to be an important variable in relation to setting times T_i and T_f (Figs. 2 and 3). Concentration is correlated with T_i, however the relationship is the inverse of that which would be expected. It would be expected that higher concentrations would have shorter setting times, as a consequence of an increased collision frequency between the polyacid molecules and the Ca^{2+} ions. However as concentration decreases, especially in the case of the D-R50 and G-E9 containing cements, initial setting times also decrease. Although the final setting time appears to increase with decreasing concentration of the polyacid, this may be an artefact caused by the brittleness of certain cement formulations. One cement, made with a 10% concentration of D-R50 proved to be too brittle for accurate determination of T_f and it is thus absent from the final setting time graph (Fig. 2). This brittleness was particularly pronounced in cements containing low concentrations of D-R50, but this was also true of some cements made with low concentrations of the G-E7 and G-E9 making it difficult to determine T_f. In the case of the G-E7 containing cements specifically, intermediate concentrations (20%) result in the longest T_i and T_f. The longest T_i observable is found using a 20% concentration of the PAA, after which the aforementioned positive correlation between concentration and T_i becomes evident. The effect of this intermediate concentration is likely to be as a result of a complex process, which is discussed later.

Mechanical properties

The D-R50 containing cements exhibited inferior properties in compression, biaxial flexure and diametral tensile strength in comparison to the G-E7 and G-E9 containing cements and were relatively invariant over the concentration range investigated, ANOVA showed no significant difference between any of the values recorded for D-R50 containing cements at any concentration for all three mechanical tests (Figs. 4–6). A 10% concentration of D-R50 resulted in cements that were too weak to extract from their molds without considerable damage, which accounts for the lack of strength values for this formulation in compressive and biaxial flexural tests. However, the influence of concentration is more apparent in the higher molecular weight PAA containing cements. All mechanical test results suggest that intermediate concentrations of the G-E9 and G-E7 tend to lead to the

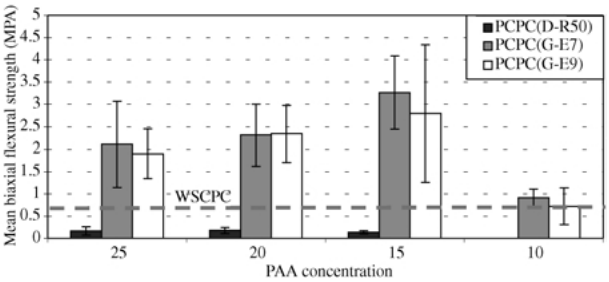

Figure 6 BFS of the PCPCs.

strongest cements, 20% in respect to compressive strength, and 15% in terms of DTS and BAFS. An ANOVA analysis shows that in the case of the compressive strength (Fig. 4) data, that 20% concentrations of G-E9 and G-E7 are statistically significantly stronger than both 15% and 10% concentrations. The diametral tensile strengths of both G-E9 and G-E7 at the 20% and 15% concentrations are significantly stronger than all other concentrations investigated, within each of the molecular weight groups (Fig. 5).

Discussion

The setting reaction of the WSCPC cement is different from that of PCPC. The presence of PAA in a CPC results in a cement setting reaction that is more akin to a glass-ionomer or zinc polycarboxylate cement [16, 17]. Calcium ions are released from the TTCP via neutralization of the hydrogen ions released from the PAA; these are then cross-linked by PAA molecules leading to setting via the formation of a calcium polyacrylate network. A secondary reaction is believed to occur between the residual calcium and phosphate ions which combine and precipitate HA out of solution, much as happens in the WSCPC [16]. It is important to note that DCPA does not participate directly in the polymeric setting reaction, however DCPA contributes to the provision of calcium and phosphate ions for HA production and secondly it is likely to encourage the epitaxic growth of any HA crystals formed. A water setting CPC similar to that proposed by Brown and Chow [1] was prepared and tested under identical conditions to the PCPCs, to allow comparisons to be made. The particle size of the TTCP in the WSCPC formulation differs from that of the PCPCs produced in this study as optimization of particle size led to the selection of a larger particle size for the PCPC specifically as smaller particle sizes reacted too rapidly to form a cement. Hence the properties of the two types of cements have been compared to show that the PCPCs can posses superior or comparable mechanical properties to a WSCPC, with the lack of initial solubility, a problem that afflicts WSCPCs in general.

The influence of molecular weight of PAA on cement properties

The influence of the choice of PAA on T_i and T_f could be related to 2 factors. First, these results may be as a consequence of the length of the PAA chains and the influence of this on mechanical properties. The methodology by which the Gillmore tests works is that of resistance to penetration as a means of determining setting time, thus the rate at which cements become stronger, which itself relates to molecular weight is likely to effect both T_i and T_f. The second possibility is that the differences in setting times are not directly related to the molecular weights of the polyacrylic acids but to other properties, particularly pH. The pH of the PAA may be important as TTCP is basic and thus the rate at which it dissolves is related to the pH of the cement liquid, this will also increase the rate of release of calcium ions and thus in theory the formation of polyacrylate cross-links. However, the G-E9 and G-E7 have comparable pH values, and G-E9 containing cements still have shorter initial and final times than their G-E7 containing counterparts, suggesting that although pH may be a factor, that molecular weight is a determinant of working and setting times.

The molecular weight of the polymer is likely to influence the compressive strength and DTS since it is related to the length of the polymer chains as observed in relation to GICs [21–23]. The higher the molecular weight of the PAA the longer the polymer chains and even in a situation where no cross-linking may occur, longer chains are likely to convey higher strength due to lower mobility and a greater ease of packing. Some values reported for the mechanical tests are weaker than those reported for the water setting system, although possibly as a consequence of the larger TTCP grain sizes used, it is also likely to be as a consequence of inhibition of the water setting system by the PAA. Previous investigations using PAAs [18] have observed similar phenomena; inhibition is most likely to be due to adsorption of PAA onto the deposited HA [24] inhibiting crystal growth and interlock, which normally conveys strength in the water-setting reaction [11].

The influence of concentration of PAA on cement properties

The decrease in T_i with decreasing concentration, in the case of the D-R50 and G-E9 containing cements is likely to be as consequence of the viscosity of the cement liquid. A similar explanation exists in relation to the T_i of the G-E7 containing cements, however in this case it is likely that two different processes are occurring. Although lower concentrations are likely to lead to less cross-link formation and thus lower mechanical strengths, as was observed; the resultant decrease in viscosity as a consequence of dilution may more than compensate for this, as relates to T_i. The effect of viscosity in these polymeric cements may be as a consequence of two separate reasons or a combination of both. First, high viscosity reduces dissolution and in the case of the coarse TTCP grains, may limit the release of calcium ions that are required to form the polyacrylate. Second, once the TTCP has been dissolved the mobility of the released calcium ions will be reduced as viscosity increases reducing the potential for cross-link formation.

The reduced T_i of the 25% G-E7 containing cements relative to the 20% G-E7 containing cements may be a consequence of a complex interaction between viscosity and concentration. As mentioned earlier, high concentrations are expected to lead to a short T_i as a consequence of a greater collision frequency between the PAA molecules and the Ca^{2+} ions; however, a lower viscosity by dilution may be able to compensate for this. It is possible therefore that at 20% PAA concentration the collision frequency is neither high enough, nor the viscosity low enough to allow a fast rate of setting by either of the two mechanisms stated. Such a mechanism may also exist with respect to the G-E9 cements, however, the fast setting reaction coupled with the methodology used might make it difficult to observe such phenomena.

The negative correlation between T_f and concentration can be explained in two ways. First, the viscosity of the PAA may no longer remain an important parameter since the viscosity increases with setting regardless of the initial viscosity of the cement liquid. As the setting reaction proceeds, even formulations with a low viscosity cement liquid will attain high viscosity due to the setting reaction. It is likely that in this high viscosity state, movement of ions and dissolution is limited in all formulations and that concentration and thus collision frequency become greater determinants of setting time than viscosity of the cement liquid. The reliance of this process on the viscosity of the cement, would explain why it is apparent in regard to T_i and not T_f. Secondly the brittle nature of these cements can lead to erroneous measurements of T_f since the use of the heavy Gillmore may damage the brittle surface.

With regard to the mechanical tests used, the lack of variation in the effect of the concentration of D-R50 in the cements might relate to the very small values obtained, or to the molecular weight of the D-R50. It is possible that the D-R50 chains are so short that even when cross-linked together they do not convey much strength to the cement, to such an extent that their number (determined by concentration) is largely irrelevant.

The increased strength of 20% (CS) and 15% (DTS/BFS) G-E9 and G-E7 containing cements, relative to other concentrations, may exist for a number of reasons. The most plausible reason is that a relatively lower concentration (20% or 15%) of the PAA would decrease the rate of formation of the insoluble polyacrylate and thus encapsulation of the precursors [17, 18], allowing better precursor dissolution relative to the 25% containing cements and the liberation of more Ca^{2+} ions for cross-linking. These cements would then be superior to even lower concentrations due to the increased number of polymer chains.

Conclusion

In conclusion, both the molecular weight and concentration of the PAA additive have an effect on the initial and final setting times, compressive strength, diametral tensile strength, and the BFS of the PCPC. Some PAAs, depending on their molecular weight and concentration can improve the properties of the TTCP/DCPA cement formulation, when used as the cement liquid. However, lower concentrations or lower molecular weight PAAs, can produce cements with inferior physical properties relative to the WSCPC, coupled with comparably long working and setting times in some cases.

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