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Development of an apatitic calcium phosphate cements: effect of liquid/powder ratio on the setting time.

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Abstract

Calcium phosphate cement (CPC) sets in situ to form resorbable hydroxyapatite with chemical and crystallographic similarity to the apatite in human bones, hence it is highly promising for clinical applications. Among the clinical requirements for calcium phosphate bone cements are initial setting time and final setting time. α-tricalcium phosphate (α-TCP) and hydroxyapatite (HA) were mixed with dicalcium phosphate dehydrate (DCPD) to form the cement powder which is mixed with aqueous solutions of 3% Na2HPO4.2H2O in weight at four different liquid-to- powder ratios (0.35, 0.40, 0.45 and 0.50 mL/g). The cement powder, on wetting with the medium, formed a workable putty. X-ray diffraction (XRD), Energy dispersive X-ray spectroscopic (EDS), transmission electron microscope (TEM) and scanning electron microscopy (SEM) techniques were employed to evaluate the phase composition and surface morphology of the cements. The results revealed similar phase composition for all samples before and after soaking in distilled water at 37°C. According to the results, it is shown that almost complete transformation of cements in calcium-deficient hydroxyapatite (CDHA) occur after soaking 7 days in destilled water with nanosized rod-like hydroxyapatite crystals. Also by reducing the L/P ratio from 0.50 to 0.35, initial and final setting times of the CPCs decreased 11 and 10 minutes respectively..

Keywords: *Calcium phosphate cement, hydroxyapatite, setting time.*

1.Introduction

Development of calcium phosphate cements (CPC) as a class of viable biomaterials has increased interest since 1980s [1], as substitutes for bone tissues and as a fixation material between ceramics and natural bone tissues in clinical applications such as orthopedic, reconstructive, oral and maxillofacial surgery [1]. There are significant advantages in application of these cements since they selfsetting under ambient or human body temperature, offer the surgeon moldability, injectability, complete filling of a cavity within the operating theatre and suitable mechanical strength in acceptable clinical time [2]. Calcium phosphate cements can be molded and self harden in the prepared bone site to form resorbable hydroxyapatite [3]. The first self-setting calcium phosphate cement, referred to as CPC, was developed in 1986 [3]. Since then, many compositions of calcium phosphate cements have been formulated and tested [4-8]. Generally, a CPC formulation contains calcium and phosphorous based ingredients in powder form, which on mixing with an aqueous medium forms a workable, self setting putty [9]. The ingredients dissolve in the medium making it supersaturated with a desired calcium phosphate which gets reprecipitated inside the mass [10]. The growth of the calcium phosphate phase as entangled crystallites, helps the putty to retain its strength and shape [11]. The design of a CPC is done on the basis of the calcium–phosphorous (Ca/P) ratio of the final precipitate and the physicochemical characteristics of precipitation (setting time, hardness, chemical stability, etc). At least two reactants are necessary, with differing Ca/P ratios adjusted to obtain the desired phase. There are two principal CPCs : apatite (HA and CDHA) and brushite cements (DCPD). Many different CPCs formulas have been studied, but most of them form HA as final product [12]. On the other hand, for the liquid part, a variety of solutions have been applied in the formulations, including water [13], citric acid aqueous solution [14], Na_2HPO_4 aqueous solution [15], H_3PO_4 [16], etc. The properties of CPCs are affected by many factors. Therefore, it is imaginable that all the factors, such as the chemical composition of the cement, the relative proportions of the reactants in the mixture, powder or liquid additives acting as accelerators or retarders, particle size, liquid / powder ratio, pressure applied during sample preparation and aging conditions, will affect its properties [17]. Many of the studies directed to improving CPC properties for clinical applications. Most of them were focused on improving next points: processing techniques [17], CPC mechanical properties [18], influence of CPC material properties on cell behavior [19], CPC materials as drug delivery systems [20, 21], stem cell delivery by CPC [21]. The improvement of these properties is carried out by introducing a different polymer, fiber or ceramic additives in CPC based on brushite or apatite. In this study, we proposed to design new apatitic CPCs formulation which have a Ca/P molar ratio values belowe than 1.5 using α- TCP 70 wt.%, DCPD 20 wt.% and HA 5 wt.% as ingredients and to investigate the effect of liquid-to-powder ratio on the phase formation, microstructure and setting properties.

1. Materials and methods

1.1. Experimental

α-tricalcium phosphate, nanocrystalline hydroxyapatite and dicalcium phosphate dihydrate were synthesized and characterized as described in our previous publications according to methods described in [22], [23] and [24] respectively. CPCs were prepared manually by mixing a powder phase with a liquid phase at four different liquid-to-powder (L/P) ratios (0.35, 0.40, 0.45 and 0.50 mL/g). By varying the ratio L/P, four CPCs were obtained, marked as CPCX with X the value of ratio L/P. The powder phase was α-Tricalcium Phosphate (70 wt.%) and HA (5 wt.%) with 20 wt% DCPD. The liquid phase consisted in aqueous solutions of 3% $Na_2HPO_4.2H_2O$ in weight. Powder phase components were placed into an agate mortar, a volume (ml) of the aqueous solution of disodium hydrogen phosphate is dropped onto the powder and the mixture is kneaded with an agate pestle for 2 min thoroughly to form a homogenous paste. The so-called Gillmore needles are suitable to measure the setting times of CPCs. The light and

thick needle is used to measure the initial setting time, t_i, the heavy and thin needle for the final setting time, t_f [25]. The clinical meaning of t_i is that it indicates the time from where the paste may not be deformed without damaging the structure of the solidifying cement. That of t_f indicates the time from when the cement can be touched without scratching it. These parameters are important because the cement must be applied before t_i and the wound may be closed after t_f. Each sample was immersed individually in a closed container contains deionised water (DI). Samples were stored at 37°C in an incubator for 1, 7, and 30 days.

1.2. Characterization

The phase composition of the cements before and after soaking in DI solution was checked by X-ray diffraction (XRD) using a Diffractometer system XPERT-3 PW3050/60 in a theta-theta setup with Cu-Kα irradiation, nickel filter. Diffraction patterns were collected between angles (2θ) of 5–60°, in steps of 0.02° with 5 s per step. The morphologies of samples were characterized by a TECNAI G2/FEI transmission electron microscopy (TEM), operating at an accelerating voltage of 200 kV. The surface changes of samples ultrastructure and morphology were examined with a scanning electron microscope (ESEM, Quanta 200 FEI) at operating voltage of 15kV, with EDAX Energy Dispersive System for X-ray analysis using an accelerating voltage of 10 kV.

2. Results and Discussion

Figure 1 shows the cements setting times as a function of L/P ratio. The setting times of different cements are summarized in Table 1. Of note, an increase in the L/P ratio resulted in an increase of both initial and final setting times of the cements. The setting time of CPCs0.5 (t_i = 28min; t_f = 37min) and CPCs0.45 (t_i = 21min; t_f = 35min) is much shorter than that of CPCs0.4 (t_i = 18min; t_f = 28min) and CPCs0.35 (t_i = 17min; t_f = 27min) in both the initial and final settings.

Table 1. The initial and final setting times of CPCs

Sample	CPCs0.35	CPCs0.4	CPCs0.45	CPCs0.50
Initial setting time (min)	17	18	21	28
Final setting time (min)	27	28	35	37

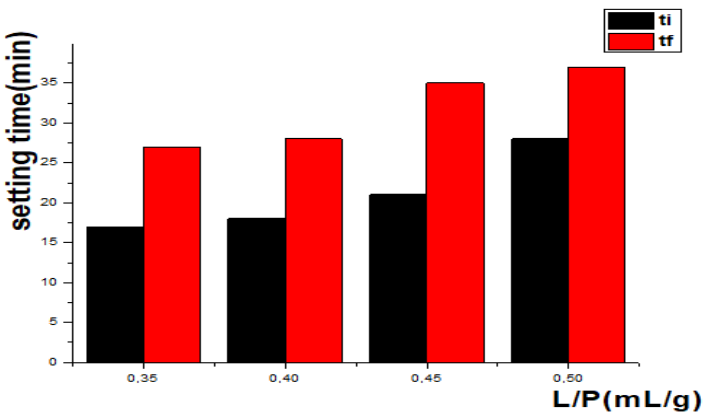


Figure 1. Initial (ti) and final (tf) setting time of CPCs prepared at different L/P ratios

The XRD patterns of cements before and after soaking in DI at 37°C for 1 and 7 days at L/P ratios of 0.35, 0.40, 0.45 and 0.50 mL/g are shown in Figure 2. Comparing XRD analysis of CPCs prepared at four different L/P ratios showed that all of them had similar patterns and this means that change in L/P ratio had no effect on the final phases of CPCs. XRD analysis showed that the conversion of cement components into hydroxyapatite occurred and its intensities increased with soaking time. Before soaking samples in water (0 day) the hardened cements composed of α-TCP, DCPD and HA which are closely similar to the cement powder. As known, the concentration of HA in cements composition is about 5 wt% and this could not be detected by XRD technique and thus the peak related to HA relates to the HA product resulted from setting reaction. After 1 and 7 days of soaking, the diffraction peaks of hydroxyapatite with increased intensity comparing to unsoaked samples are observed (at 2θ=26, 32 and 33). Nevertheless, some α-Tricalcium phosphate, initially introduced, remained in the final composition. Moreover, CPCs0.5 has higher extent of HA transition than others.

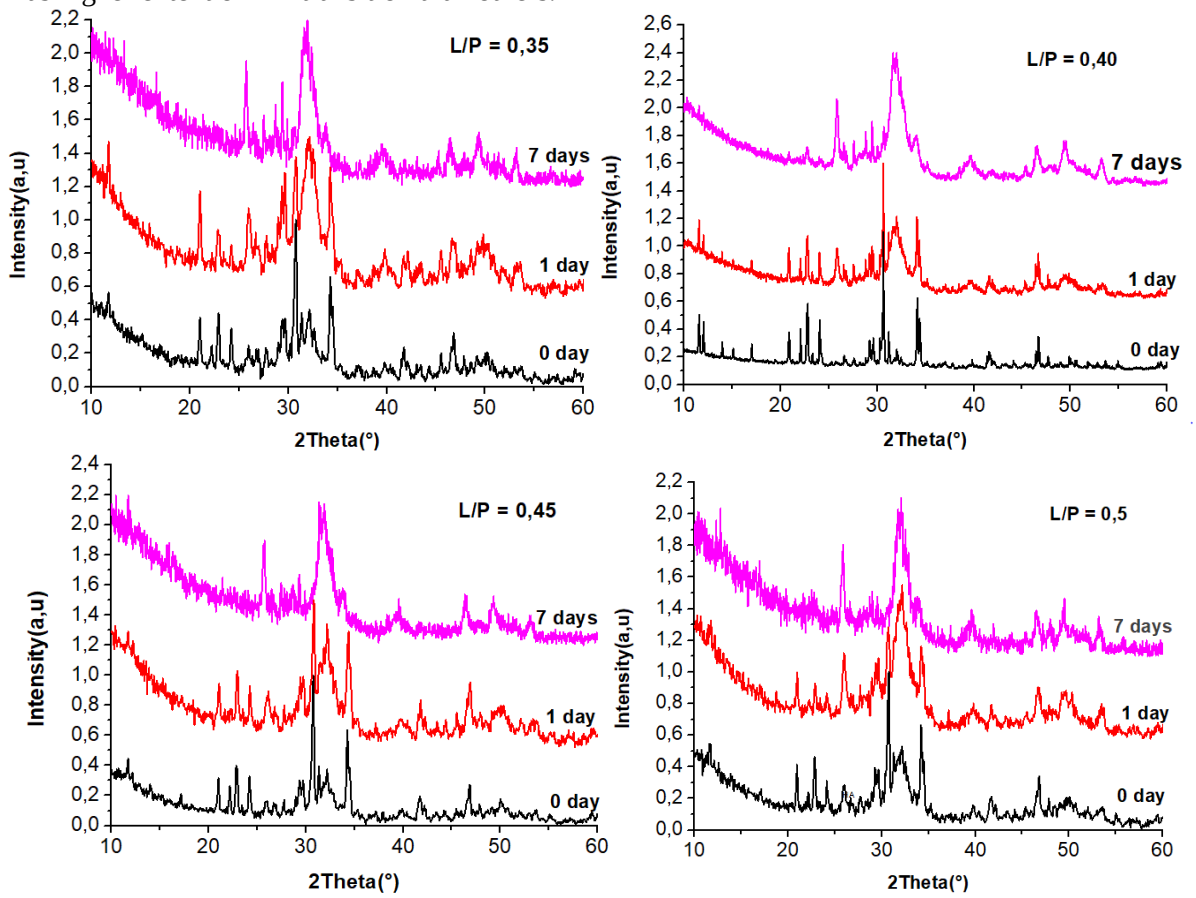
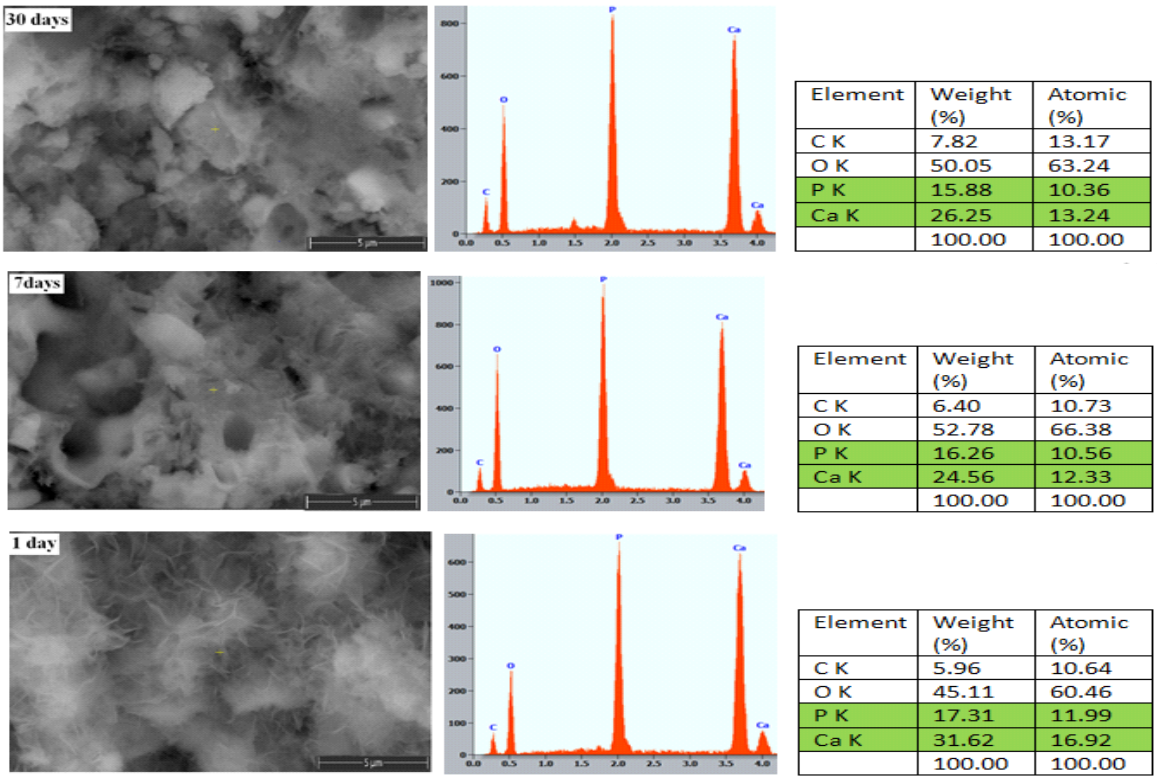


Figure 2. X-ray diffractogram of CPCs prepared at different L/P ratios after soaking in DI for 0, 1 and 7 days

SEM analyses revealed surface precipitate with ultrastructure and morphology changes corresponding to the soaking time in DI for all CPCs. SEM micrographs of fracture surfaces of CPC0.35 specimens are shown in Figure 3 at 1, 7 and 30 days soaking time. After 1 day, we observed the formation of spherical and globular precipitates with acicular crystallites (spike-like structure) peripherally and over the samples surface. After 7 days, more globular precipitations homogeneously covered the surface with a well developed spherical morphology and mesoporous structure. After 30 days, the deposition layer thickened and appeared significantly more porous. EDX analysis revealed the presence of mainly calcium and phosphorus ions in the precipitations indicating the formation of calcium phosphate depositions since day one. Ca/P molar ratio of precipitations decreased from 1.41 on day 1 to 1.27 on day 30 corresponding to the representative micrograph in Figure 3 for the field of view.

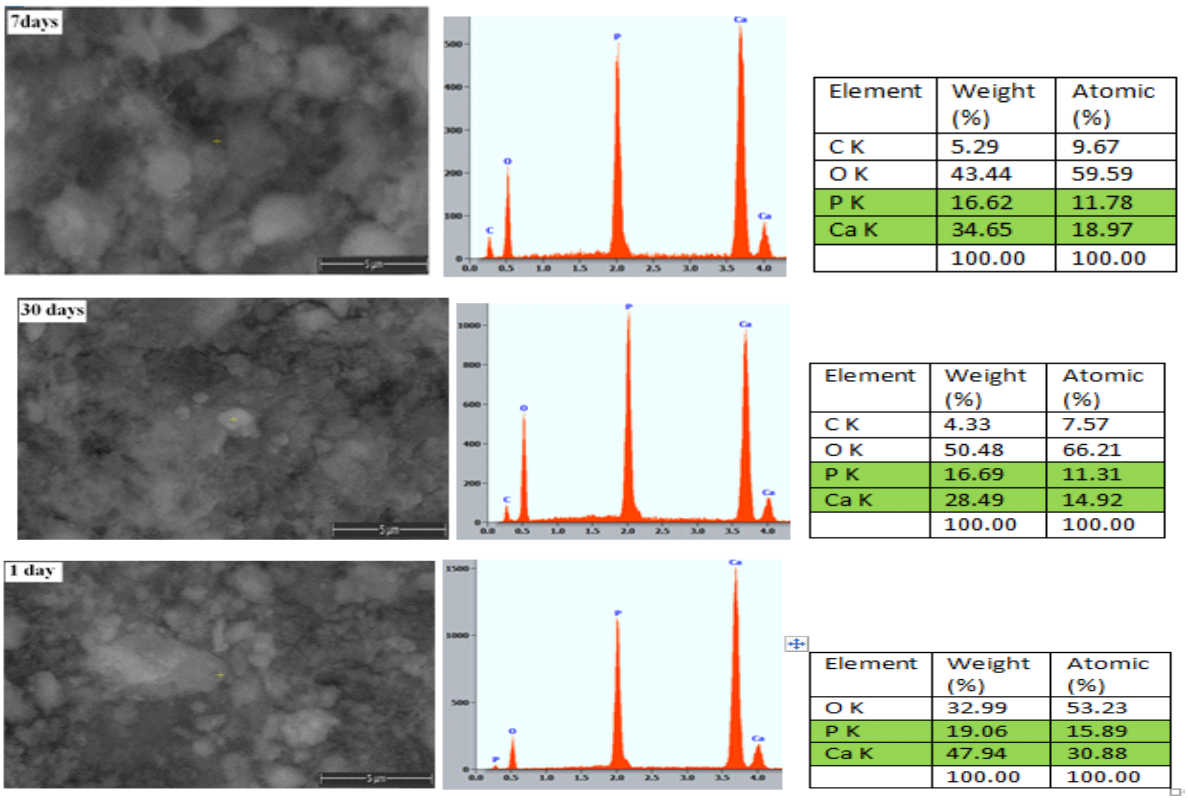


Element	Weight (%)	Atomic (%)
C K	7.82	13.17
O K	50.05	63.24
P K	15.88	10.36
Ca K	26.25	13.24
	100.00	100.00

Element	Weight (%)	Atomic (%)
C K	6.40	10.73
O K	52.78	66.38
P K	16.26	10.56
Ca K	24.56	12.33
	100.00	100.00

Element	Weight (%)	Atomic (%)
C K	5.96	10.64
O K	45.11	60.46
P K	17.31	11.99
Ca K	31.62	16.92
	100.00	100.00

Figure 3. SEM micrographs of the surface of CPCs samples prepared at L/P = 0.35. EDX spectrum was obtained from the precipitates in the field of view. Semiquantitative chemical composition is presented in the table



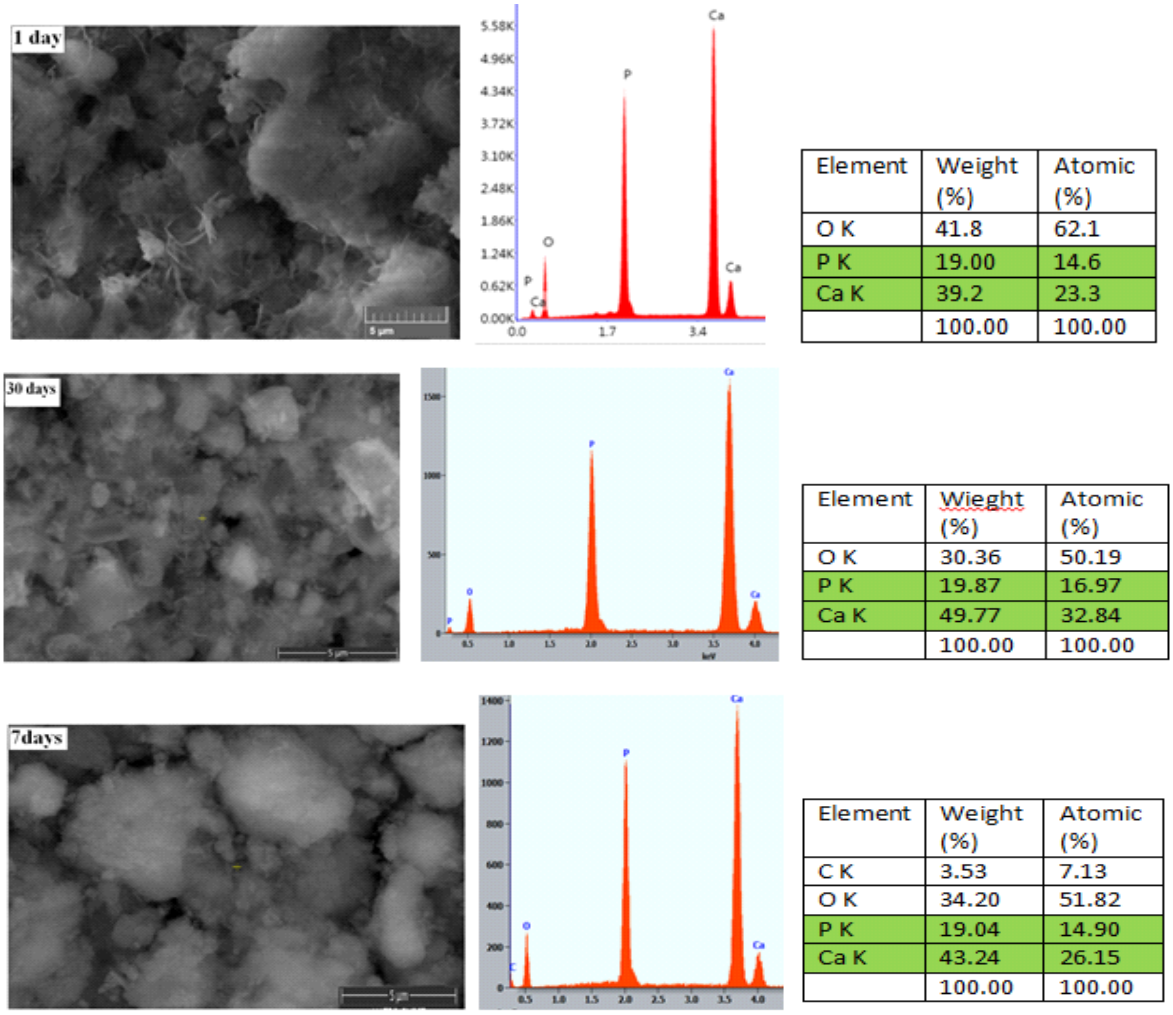
Element	Weight (%)	Atomic (%)
C K	5.29	9.67
O K	43.44	59.59
P K	16.62	11.78
Ca K	34.65	18.97
	100.00	100.00

Element	Weight (%)	Atomic (%)
C K	4.33	7.57
O K	50.48	66.21
P K	16.69	11.31
Ca K	28.49	14.92
	100.00	100.00

Element	Weight (%)	Atomic (%)
O K	32.99	53.23
P K	19.06	15.89
Ca K	47.94	30.88
	100.00	100.00

Figure 4. SEM micrographs of the surface of CPCs samples prepared at L/P = 0.45. EDX spectrum was obtained from the precipitates in the field of view. Semiquantitative chemical composition is presented in the table

The micromorphology of CPC0.45 and CPC0.5 is shown in Figure 4 and 5 respectively. Precipitations in the form of globular aggregates with both acicular and smooth morphology were observed after 1-day soaking in DI. After 7 days, the whole area is covered with small globular depositions that homogeneously covered the cement surface (figure 4 and 5). EDX analysis illustrated majorly calcium and phosphorus ion supporting the formation of calcium phosphate. Ca/P molar ratios of deposition in the field of view in CPC0.45 (Figure 4) and CPC0.5 (Figure 5) after 1 day of aging were 1.94 and 1.60, respectively, decreasing to 1.32 in CPC0.4 and increasing to 1.94 in CPC0.5 after 30 days soaking in DI.



Element	Weight (%)	Atomic (%)
O K	41.8	62.1
P K	19.00	14.6
Ca K	39.2	23.3
	100.00	100.00

Element	Wieght (%)	Atomic (%)
O K	30.36	50.19
P K	19.87	16.97
Ca K	49.77	32.84
	100.00	100.00

Element	Weight (%)	Atomic (%)
C K	3.53	7.13
O K	34.20	51.82
P K	19.04	14.90
Ca K	43.24	26.15
	100.00	100.00

Figure 5. SEM micrographs of the surface of CPCs samples prepared at L/P = 0.50. EDX spectrum was obtained from the precipitates in the field of view. Semiquantitative chemical composition is presented in the table

Transmission Electron Microscopy (TEM) was also employed to investigate the size and morphology of CPCs nanoparticles. Results from TEM analyses for CPCs nanoparticles soaked 7 days in DI for various L/P ratios are shown in Figure 6. It reveals that the CPCs particles have a rod-like morphology with sizes ranged from 40 to 100 nm in the long axis and from 10 to 18 nm in the short axis. However, the CPCs nanoparticles exhibited high tendency to agglomerate. It can also be seen that by increasing the L/P ratio, particles size increases.

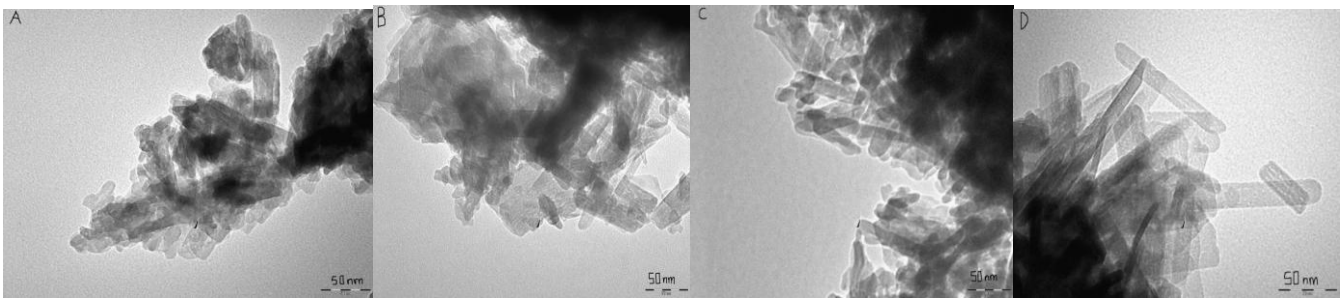


Figure 6. TEM images of CPCs prepared at L/P ratio of (A) 0.35, (B) 0.40, (C) 0.45, (D) 0.50

The fundamental solidifying mechanism of the calcium phosphate cements is the dissolution–reprecipitation reaction based on difference in thermodynamic stabilities of calcium phosphate salts.

By mixing the powder phase of CPCs with liquid phase, the so-called dissolution–reprecipitation reaction occurs. At pH of higher than 4.2, hydroxyapatite is the most stable phase thermodynamically. Hence, HA crystals are formed when appropriate calcium phosphate salts are mixed with the liquid phase to make the pH either neutral or alkaline.

The major component of powder phase in this study was α-TCP which is an unstable phase in water. In other words, α-TCP dissolves to supply calcium and phosphate ions. However, the solution is supersaturated with respect to the HA. Therefore, calcium and phosphate ions will be precipitated as calcium deficient hydroxyapatite (CDHA) crystals. So, the final result of the hydration reactions is a hardened mass with high strength. The use of higher amounts of liquid in the cement (higher L/P ratios) increases the working time of the mass which delays the supersaturation of hydrate phases, which in apatite CPCs is expected to be hydroxyapatite, and this leads to longer setting times of the cements.

3. Conclusion

The results of the present study suggest that all the cements possess the ability to form apatite precipitation in deionised water. The formed apatite layer in all the cements can thicken as the function of time. It can be concluded that reduction of liquid-to-powder ratio leads to decrease both their initial and final setting time. Moreover, the variation of this ratio has no effect on the final phase composition of cements. A rod-like morphology cement with smaller size can be obtained by decreasing the liquid-to-powder ratio.

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