EFFECT OF POLY (ETHYLENE GLYCOL) ON THE INJECTABILITY, SETTING BEHAVIOR, AND MECHANICAL PROPERTIES OF CALCIUM PHOSPHATE BONE CEMENT

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ABSTRACT: The present paper reports on the effect of poly(ethylene glycol) (PEG) addition on injectability, setting behaviour, and mechanical properties of calcium phosphate cement (CPC) for injectable bone filling applications. Calcium hydroxide, $Ca(OH)_2$, and diammonium hydrogen phosphate, $(NH_4)_2HPO_4$, have been used as precursors in wet chemical precipitation synthesis of hydroxyapatite powder. Cement paste was prepared with different powder-to-liquid ratios, varied at 1.0, 1.3, 1.5 and 2.0. The incorporation of PEG was also varied at 1, 2, 3, 4 and 5 wt% at the powder-to-liquid ratio of 1.3. The CPC produced was then evaluated in terms of injectability, setting time and mechanical strength. The results indicated that PEG addition significantly improved setting time, injectability, as well as compressive strength of CPC. Without PEG, the initial setting time ranged between 3 and 122 min, while the final setting time ranged between 5 and 277 min. The addition of PEG has significantly improved setting time where the initial setting time ranged from 47 to 88 min and the final setting time ranged from 182 to 228 min. The extrusion load decreased when PEG was added, which revealed an improvement in injectability; 82.5% without PEG addition and 95.5% when 5% PEG was added. The compressive strength of CPC is in the range of 0.59 to 1.344 MPa and its porosity is in the range of 39.2% to 47.1%. With the incorporation of PEG, the compressive strength greatly increased to the range of 1.167 and 1.786 MPa..

ABSTRAK: Penyelidikan ini melaporkan tentang kesan menambah polietilina glikol (PEG) terhadap sifat-sifat simen kalsium fosfat seperti keupayaan suntikan, masa pengerasan dan kekuatan mekanikal. Kalsium hidroksida dan diammonium hidrogen fosfat digunakan sebagai reagen dalam kaedah pemendakan kimia basah bagi menghasilkan serbuk hidroksiapatit. Pes simen disediakan dengan nisbah serbuk kepada cecair yang berbeza, dengan nisbah 1.0, 1.3, 1.5 dan 2.0. Kemudian, PEG ditambah ke dalam simen kalsium fosfat dengan kepekatan yang berbeza, bernilai 1, 2, 3, 4 dan 5 wt% bagi 1.3 nisbah serbuk kepada cecair. Simen yang terhasil diuji bagi menilai keupayaan suntikan, masa pengerasan dan kekuatan mekanikal. Keputusan ujian-ujian tersebut menunjukkan bahawa penambahan PEG ke dalam simen telah meningkatkan kebolehan simen untuk disuntik, mengurangkan masa pengerasan simen dan meningkatkan kekuatan

mekanikal simen. Simen tanpa PEG mempunyai masa pengerasan awal daripada 3 min kepada 122 min dan masa pengerasan akhir daripada 5 min kepada 277 min. Apabila PEG ditambah, masa pengerasan simen menjadi lebih baik dengan masa pengerasan awal daripada 47 min kepada 88 min dan masa pengerasan akhir daripada 182 min kepada 228 min. Peningkatan dalam keupayaan simen untuk disuntik telah dibuktikan dengan pengurangan beban penyempitan apabila PEG ditambah ke dalam simen. Simen tanpa PEG mempunyai 82.5% keupayaan suntikan dan meningkat kepada 95.5% apabila 5% PEG ditambah. Kekuatan mampatan simen bernilai antara 0.59 dan 1.334 MPa dan keliangan simen bernilai antara 39.2% dan 47.1%. Kekuatan mampatan simen meningkat dengan ketara apabila PEG ditambah, bernilai antara 1.167 dan 1.786 Mpa.

KEYWORDS: *calcium phosphate cement; injectability; polymeric additives; wet chemical precipitation method*

1. INTRODUCTION

Autograft and allograft are standard procedures in bone grafting to repair bone defects. However, bone grafting has a number of limitations including inadequate bone supply and requiring additional operations for autograft, while there is high risk of immunological reactions and disease transmission in allograft [1,2]. Development of synthetic biomaterials has been an alternative to overcome the limitations of bone grafting; bone cement has been attracting considerable attention as human hard tissue filler and joint anchorage materials since the second half of the last century.

Clinically available bone cement materials are polymethylmethacrylate (PMMA), calcium phosphate cement (CPC) and calcium sulfate cement (CSC). PMMA-based cement offers biocompatibility, high mechanical strength, and excellent setting and injectability. The disadvantages of PMMA-based cement are its lack of bioactivity and resorbability, high stiffness, exothermic setting reaction, as well as monomer toxicity and leakage [3]. Meanwhile, CSC has higher mechanical strength than CPC, but it degrades much faster, which causes different rates of bone regeneration and cement degradation [3].

CPC is significant and clinically accepted as injectable bone filling material. This recognition is due to its remarkable biological response, injectability, and potential to set *in vivo* [4-6]. CPC can be injected and molded to fill and take the shape of defect sites. Injectable CPC is obtained by mixing calcium phosphate powder of various phases and water at certain ratios and sets at body temperature via dissolution-precipitation mechanism [5]. Apatite cements of hydroxyapatite (HA) are formed upon mixing phases such as tetracalcium phosphate (TTCP) and α–tricalcium phosphate (α-TCP) with water [7].

Various methods have been employed to synthesize CPC, which can be classified into dry and wet methods. Dry methods include solid-state synthesis and the mechanochemical process. Meanwhile, wet methods include chemical precipitation, hydrolysis, hydrothermal, sol-gel, emulsion, and sonochemical methods [8,9]. Wet-chemical precipitation method has been widely used to synthesize CPC because it offers a simple route, low reaction temperature, highly pure end products, and inexpensive resources [10].

Clinical application of CPC is limited by poor injectability and low mechanical strength. Addition of polymeric additives is one of the approaches to enhance performance and improve properties of CPC such as injectability, setting time, cohesiveness, mechanical strength and biological response [11,12]. Several studies have addressed the use of polymeric additives including chitosan, alginate, poly(lactic-co-glycolic acid) (PLGA), poly(ethylene glycol) (PEG) and poly(vinyl alcohol) (PVA) [11].

PEG is a polyether composed of glycerol monomers. It has been used in biomedical fields because of its non-toxicity, good water solubility, flexibility, and anti-coagulant activity [13]. PEG acts as a thickening agent and keeps the cement paste stable, which has been applied to prepared premixed CPCs [11,13]. With the addition of PEG, CPC is expected to have shorter setting time, better anti-washout performance, but lower injectability [11].

The present work investigates the effect of powder-to-liquid (P/L) ratio and PEG content on the injectability, setting behavior, and mechanical properties of CPC synthesized from a wet chemical precipitation method.

2. EXPERIMENTAL

2.1 Synthesis of Powder

A wet chemical precipitation method was employed to synthesize CPC based on the procedure reported elsewhere [14]. In this case, the HA powder was prepared using calcium hydroxide, $Ca(OH)_2$, and diammonium hydrogen phosphate, $(NH_4)_2HPO_4$, as the calcium and phosphorus precursors respectively, and the solvent used was distilled water. Calcium-to-phosphate (Ca/P) ratio was fixed at 1.67. Each precursor was dissolved in distilled water to produce a calcium and phosphorus solution. Then, it was followed by drop-wise addition of a phosphorus solution into the calcium solution. 25% ammonia solution was added until pH 11 was achieved. The mixture was then refluxed at 90 ºC, followed by aging overnight at room temperature, washing with distilled water, and filtration. Afterwards, the precipitate was dried overnight in an oven at 85 ºC and, finally, crushed.

2.2 Cement Preparation

Preparation of CPC was done by mixing the as-synthesized HA powder and liquid phase at certain ratios. The P/L ratio was varied at 1.0, 1.3, 1.5 and 2.0. The P/L ratio of 1.3 was selected to prepare the CPC incorporated with PEG (MW300, Sigma) based on its optimized properties. PEG addition into the liquid phase was varied at 1, 2, 3, 4 and 5 wt%.

2.3 Powder Characterization

The wet chemical precipitation-derived powder underwent morphology and phase characterization. Phase analysis was done using X-ray Diffraction (XRD). An Empyrean, PANalytical XRD system was employed under CuKα radiation (λ = 1.5406 Å) with 2° per minute scan speed and 0.02° step size over the 2θ range of 20-60°. A Fourier Transmission Infrared (FTIR) spectrometer was employed for identification of functional groups of the sample. A Perkin Elmer Spectrum 100 FTIR spectrometer was operated over the 4000-500 cm-1 scanning range at 4 cm-1 resolution. Morphology evaluation was done by using Transmission Electron Microscopy (TEM) JEOL JEM-2100F.

2.4 Injectability Test

The extrusion method has been employed for the injectability test of CPC, in which a non-needle 5 ml polyethylene syringe was used. The extrusion of the cement paste has been done under compression mode of a Lloyd LR 10 K+ Universal Testing Machine ran at 50 mm/min crosshead speed and 300 N maximum load. Figure 1 presents the schematic diagram of the experimental setting of the injectability test. The result was plotted in graph form of extrusion force (N) against the extrusion time (s). The percentage of injectability was calculated based on equation (1).

$$\%\ injectability = \frac{mass\ of\ extruded\ paste\ from\ syringe}{mass\ of\ paste\ before\ injection} \times 100\% \quad (1)$$

The effect of P/L ratio (1.0, 1.3, 1.5 and 2.0) and PEG addition (1, 2, 3, 4 and 5 wt%) on the injectability of CPC have been evaluated.

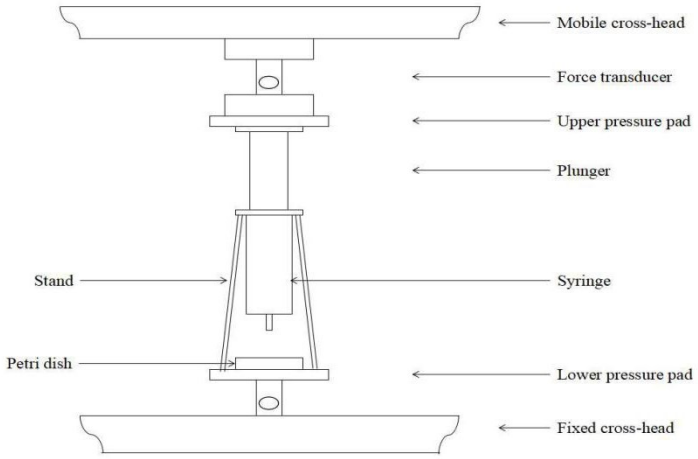

Fig. 1: Schematic diagram of the injectability test.

2.5 Setting Time

The initial and final setting times of CPC were determined using a Gillmore needle method. Powder and liquid phases were mixed for 2 min, and then the cement paste was put into a plastic mold. The effect of P/L ratio (1.0, 1.3, 1.5 and 2.0) and PEG addition (1, 2, 3, 4 and 5 wt%) on the setting time of CPC have been determined.

The determination of initial and final setting times was done by vertically indenting the thick and thin needle on the cement surface. The initial setting time is the time when no visible indentation is observed as the thick needle with a fixed low load is applied, while the final setting time is the time when no visible indentation is observed as the thin needle with a fixed high load is applied [2].

2.6 Mechanical Strength

Compression strength test has been done by using a Lloyd LR 10 K+ Universal Testing Machine operated at a crosshead rate of 1 mm/s. The cement pastes were left for 48 hours to set after molded in a Teflon mold. The sample dimension was 10 mm diameter x 15 mm length.

2.7 Porosity Measurement

The measurement of density was done using a densitometer. The resultant apparent density was then used to calculate the porosity of CPC using equation (2) and (3):

$$\%\, porosity = 100\% - Relative\ density \tag{2}$$

$$Relative\ density = \frac{\rho_{app}}{\rho_{th}} \times 100\% \tag{3}$$

where, ρ_{app} is the apparent density measured by densitometer and ρ_{th} is the theoretical density of HA, taken as 3.156 g/cm^3.

3. RESULTS

3.1 Powder Characterization

The XRD pattern of the wet chemical precipitation-derived powder is shown in Fig. 2. From the XRD pattern in Fig. 2, HA is the main phase as the peaks present are attributed to hydroxyapatite according to the standard data of the card no. 09-432. The phase purity and crystallinity degree of the synthesized powder have been confirmed by the presence of sharp and clear peaks. The TEM image in Fig. 3 shows the nanorod shape of HA particles, with the sizes of 150-300 nm length and 10-30 nm width.

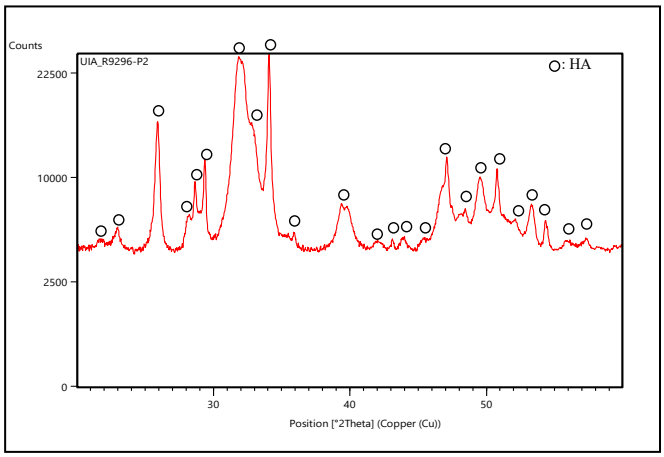

Fig. 2: XRD pattern of the wet chemical precipitation-derived powder.

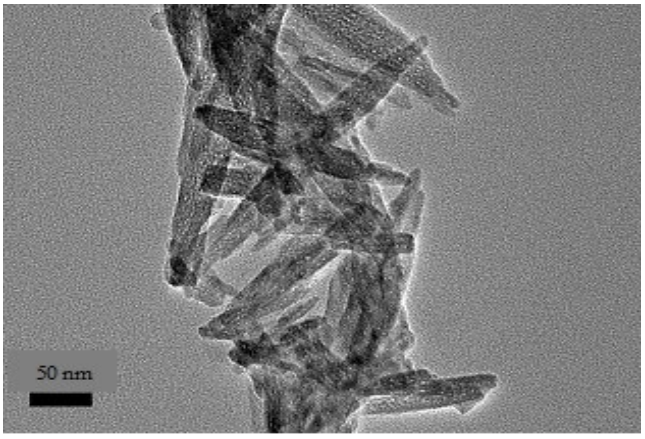

Fig. 3: TEM micrograph of the wet chemical precipitation-derived HA powder.

The existing functional groups in the as-synthesized powder were measured using FTIR and the spectrum shown in Fig. 4. The bands at 1455 cm^{-1} and 874 cm^{-1} suggest the presence of CO^{3-} in HA powder [15,16]. This might happen due to the adsorption of atmospheric carbon dioxide during the synthesis process [15,17]. PO^{4-} derived bands appear at 1025 cm^{-1}, 602 cm^{-1} and 560 cm^{-1} [15-18]. The FTIR and XRD measurements have proven the as-synthesized powders to be pure HA.

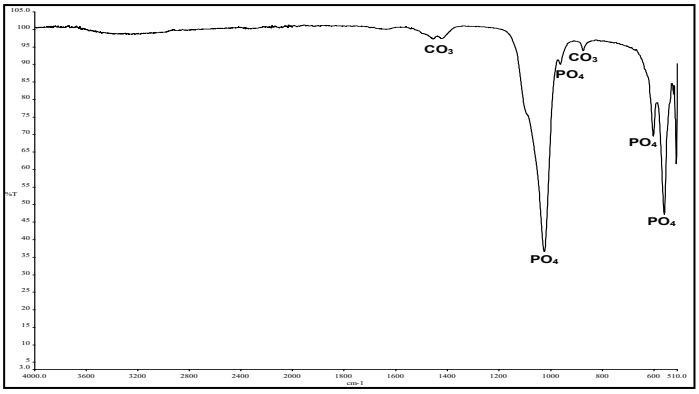

Fig. 4. FTIR spectrum of the wet chemical precipitation derived HA powder.

3.2 Calcium Phosphate Cement Characterization

3.2.1 Setting Time

CPC was prepared by varying its P/L ratios, from 1.0, 1.3, 1.5 and 2.0. Figure 5 presents that the setting time of CPC shortens with the increase in P/L ratio. Faster hardening time is attributed to the higher content of powder in the cement paste. The initial setting time ranged from 3 to 122 min, whereas the final setting time ranged from 5 to 277 min. The optimum workable setting times of CPC is achieved at the P/L ratio of 1.3, with the initial and final setting times of 88 min and 228 min, respectively.

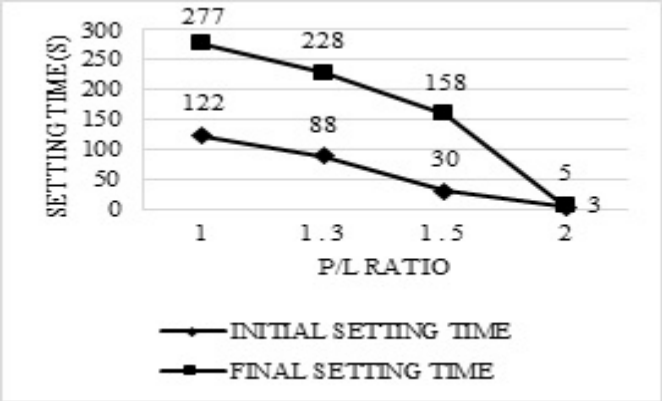

Fig. 5: Setting times of CPC with various P/L ratios.

CPC incorporated with PEG was then prepared using the P/L ratio of 1.3, and the PEG content was varied at 1, 2, 3, 4, and 5 wt%. The initial setting time is between 47 min and 88 min, whereas the final setting time is between 228 min to 182 min. Figure 6 shows the incorporation of PEG into CPC has improved setting time of CPC by shortening the hardening time of CPC. The dissolution of PEG in water formed a network structure leading to the entanglement with three dimensional apatite structures during the hydration process, and finally resulted in the acceleration of setting time [13,19]. This is true only for the addition of PEG content below 5%. The increase in both initial and final setting times were observed with the addition of 5% PEG. This result indicates that a proper amount of PEG is required to improve the setting time of CPC.

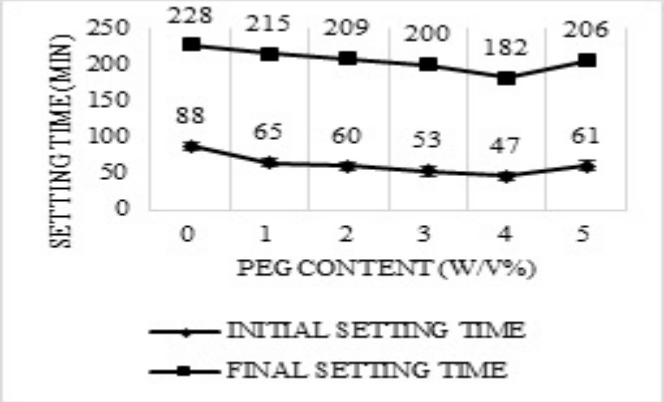

Fig. 6: Effect of PEG addition on setting times of CPC prepared with the P/L ratio of 1.3.

3.2.2 Injectability

The injectability result of CPC shown in Fig. 7 indicate that an increase in P/L ratio reduces injectability. This is due to the higher powder content which escalates the extrusion load, and hence injectability is reduced. CPC with the P/L ratio of 2.0 shows no injectability since no paste can be extruded out of the syringe. This is in good agreement with the percentage of injectability of CPC, such that it varied from 96%, 82.5%, 75.4% and 0% for P/L ratio 1.0, 1.3, 1.5 and 2.0 respectively.

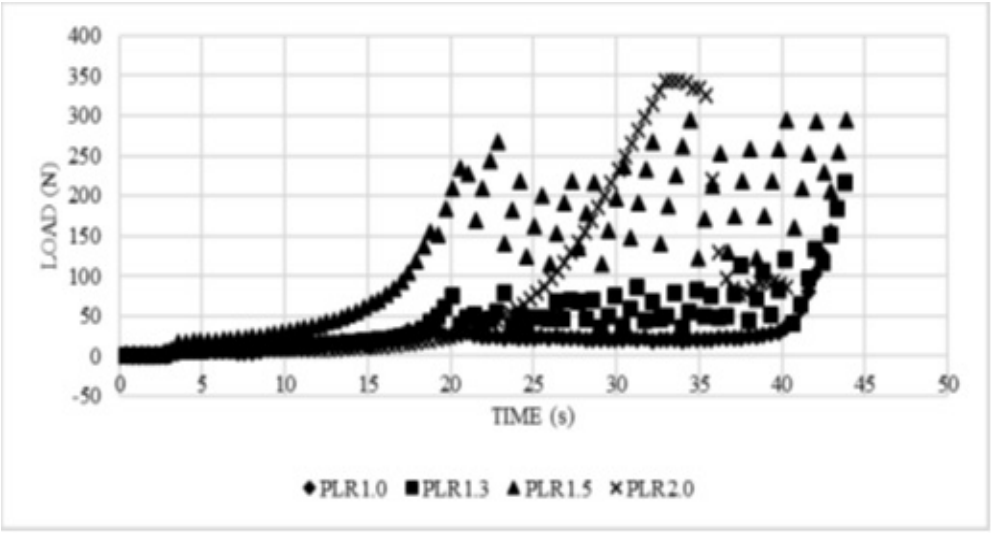

Fig. 7: Injectability of CPC with various P/L ratios.

In Fig. 8, an increase in PEG content improves injectability of CPC as the extrusion load decreased. This is supported by the results of percentage injectability, such that the injectability varied from 82.5%, 88.1%, 89.3%, 92.4%, 93.7% and 95.5% for 0, 1, 2, 3, 4 and 5% PEG content respectively. This result is in agreement with the study by Hesaraki et al. [20] that revealed significant improvement of cement paste after PEG addition as compared with paste using water only because PEG lowered the friction between CPC particles. On the other hand, Chen et al. [13] reported that injectability decreased when PEG concentration increased due to the increase in viscosity of cement paste. Hydration reaction between the PEG solution and CPC powder formed a spatial net structure via the formation of colloidal particles that join together through Van der Waals force, and hence strengthen

the network of cement paste [13]. Wang et al. [19] also demonstrated that the addition of 0.5 wt% PEG 200 decreased injectability of CPC as compared with using only water because the viscosity of the paste was higher.

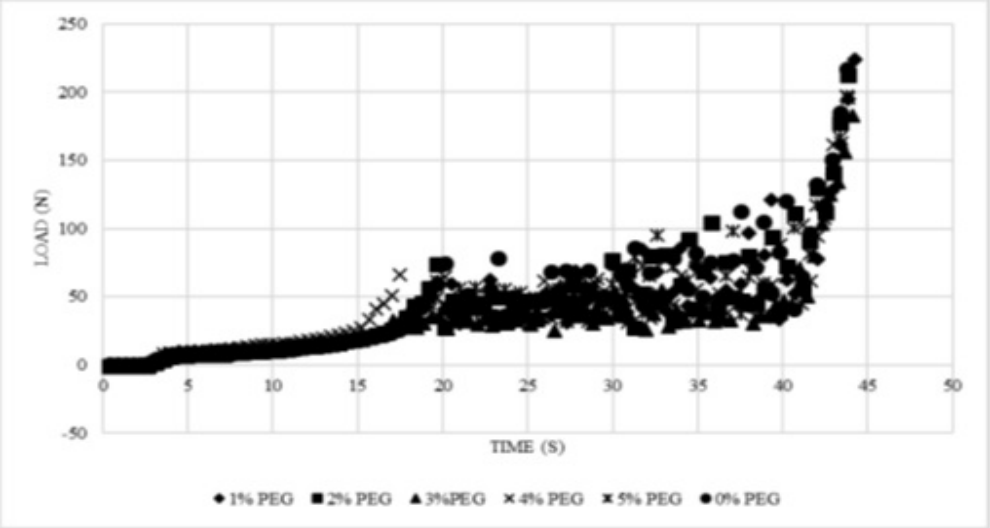

Fig. 8: Effect of PEG addition on injectability of CPC prepared with the P/L ratio of 1.3.

3.2.3 Mechanical Strength

A compressive strength test of the CPC has been done to investigate the effect of P/L ratio on the mechanical strength of CPC. Figure 9 shows the result for the compression test of CPC with different P/L ratios. The average strength of CPC without addition of PEG ranged from 0.590 MPa to 1.344 MPa. The highest strength is 1.344 MPa, achieved by CPC with the P/L ratio of 1.3. The increase in P/L ratio enhances compressive strength of CPC. However, the strength starts to decrease to 1.106 MPa and 0.621 MPa when the P/L ratios of 1.5 and 2.0 were used, respectively. This is because of insufficient water content, thus hampering the formation of apatite via dissolution-precipitation mechanisms.

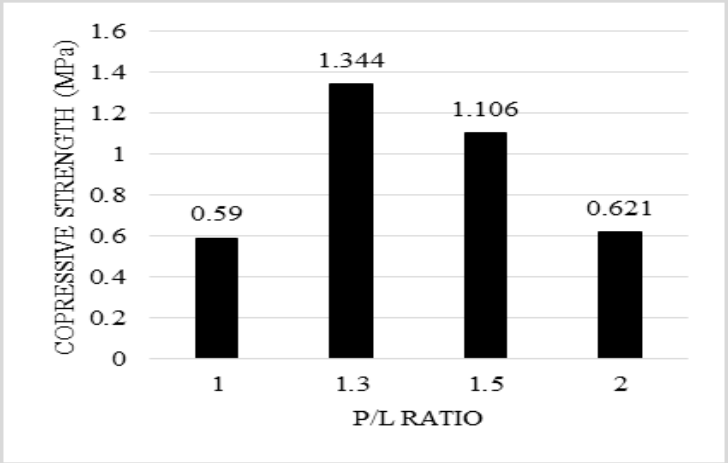

Fig. 9: Compressive strength of CPC with various P/L ratios.

When PEG is added into CPC, the strength is in the range of 1.167 MPa to 1.786 MPa. This shows the incorporation of PEG has improved the compressive strength of CPC. Figure

10 presents that CPC with 2% PEG gives the highest strength with 1.786 MPa. This result indicates that an optimal amount of PEG is required to produce CPC with better mechanical properties.

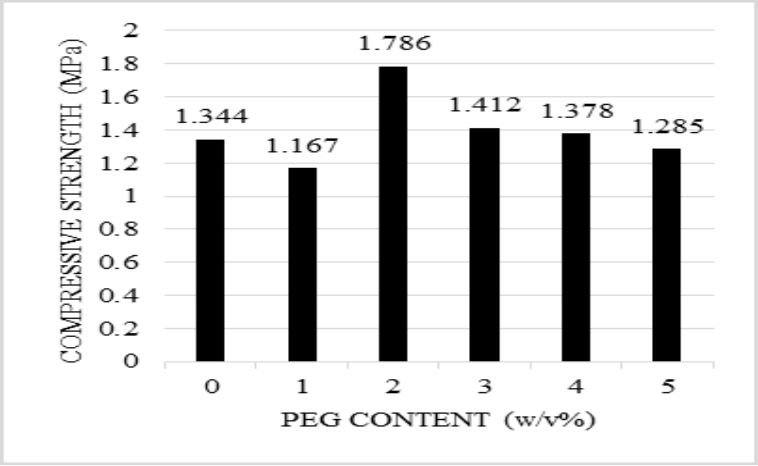

Fig. 10: Effect of PEG addition on the compressive strength of CPC prepared with the P/L ratio of 1.3.

3.2.4 Porosity

The average porosity of CPC ranged from 39.2% to 47.1%. Their apparent density ranged between 1.67 to 1.92 g/cm^3. From Fig. 11, the porosity of CPC decreases with the increase in P/L ratio. This is attributed to the decrease in water component in the cement paste. This result is in disagreement with the compressive strength of CPC. Generally, the strength improved with a decrease in porosity as the density increased. However, water content plays an important role in CPC to induce the formation of apatite crystals through the dissolution-precipitation mechanisms. Hence, the lack of water content in the cement paste might have negatively affected the strength of the cement by reducing the strength, as demonstrated by the CPC with the P/L ratios of 1.5 and 2.0 in Fig. 9.

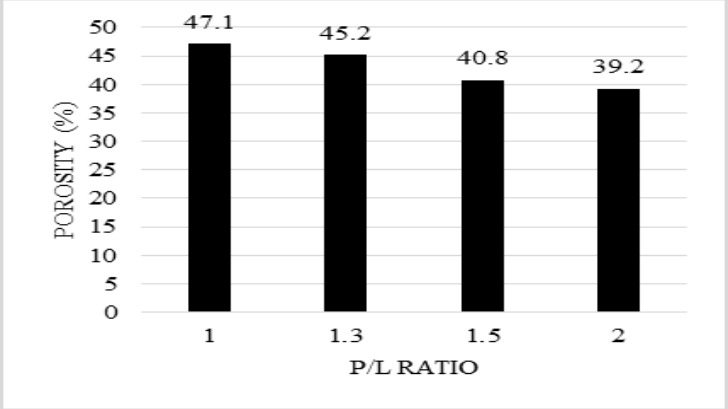

Fig. 11: Porosity of CPC with various P/L ratios.

The porosity of CPC is in the range of 39.6% to 51.4% when PEG is added and the apparent density is between 1.53 to 1.90 g/cm^3. The result shows that incorporation of PEG into CPC increases porosity of CPC as presented in Fig. 12. The higher the PEG content, the lower its porosity. This is true only for 1% to 4% PEG content. The porosity started to

increase again when 5% PEG was added. This indicates that an appropriate amount of PEG is needed to reduce porosity of CPC, and hence improves its mechanical properties.

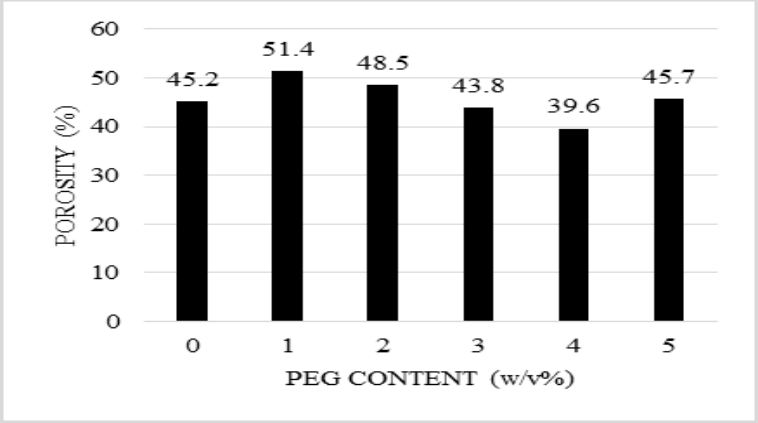

Fig. 12: Effect of PEG addition on the porosity of CPC prepared with the P/L ratio of 1.3.

4. CONCLUSION

The pure nano scale HA has been successfully synthesized through wet-chemical precipitation method using calcium hydroxide and diammonium hydrogen phosphate.

The injectability, setting behavior, and mechancial strength have been detemined to explain the effect of P/L ratio and addition of PEG on the properties of CPC. The present work reveals that high P/L ratio shortens setting time and improves mechanical strength, but reduces injectability. The optimized condition of CPC is achieved at the P/L ratio of 1.3: 88 min initial setting time, 228 min final setting time, 82.5% injectability and 1.344 MPa compressive strength.

This study has proven that addition of PEG is able to shorten the setting time, as well as enhance injectability, and mechanical strength. Incorporation of 2% PEG into CPC provides an optimum condition which revealed significant improvement in setting time, injectability, and compressive strength: 60 min initial setting time, 209 min final setting time, 89.3% injectability and 1.781 MPa compressive strength. Therefore, the incorporation of PEG into CPC could become a promising injectable bone filling material in the future.

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