

ORIGINAL ARTICLES

Influence of Impregnated Calcium Lactate Gluconate on Physical Properties and Adaptability of New formulated nano Bio-Cement of β -Dicalcium Silicate and biphasic calcium phosphate for Use as a Root Canal Sealer.

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ABSTRACT

The aim of this study is to evaluate the effect of Calcium lactate gluconate (CLG) aqueous solution on workability, setting time, dimensional stability, pH changes, calcium ion release and adaptation of β - di calcium silicate (β - C₂S) based root canal filling materials as well as root canal sealing materials. **Materials and methods:** The synthesized bio-composite composed of β -C₂S, biphasic calcium phosphate and calcium aluminate cement (CAC). CLG solution was prepared by mixing lactic acid, glucono delta lactone, and calcium oxide. The influence of various concentrations of CLG mixing liquid on initial setting time, handling properties, calcium ion release and pH value were investigated. 15 freshly extracted human lower first premolars were prepared and filled with single cone gutta percha and the tested sealer. Adaptation at the apical third was evaluated using scanning electron microscope at one week, two weeks and one month interval after immersion in distilled water. **Results** showed that by using the concentration range between 19 and 23wt% CLG solutions as a mixing liquid the setting time of the synthesized sealer decreased to a range of 17 ± 3 minutes and showed excellent handling properties of the experimental sealer. By increasing the curing period of pastes prepared with 21%CLG solution there was an increase in the pH values while the calcium ion concentration decreased. All the concentrations used in this study-exhibited expansion. The FTIR and XRD analysis revealed that the use of CLG mixing solution enhances the hydration reactions of β -C₂S phase and biphasic calcium phosphate as well as CAC. SEM results showed that there was good adaptation at one week period and decreased over time. **Conclusion:** It was concluded that Nano-particle size did influence physical characteristics of the experimental calcium phosphate cements, improved chemical reactivity, biocompatibility and decreased setting times. CLG solutions of concentration ranges from 19 to 23 wt% showed an excellent workability and acceptable adaptation.

Key words: Calcium lactate gluconate - β -dicalcium silicate - biphasic calcium phosphate - calcium aluminate cement.

Introduction

The most common cause of failure involving endodontic therapy can be attributed to the lack of an apical seal leading to leakage at the apex. Effective endodontic obturation thus, must provide a dimensionally stable, inert fluid tight apical seal that will eliminate any portal of communication between the canal space and the surrounding periapical tissues through the apical foramen (Hovland and Dumsha 1985). The sealer performs several functions during the obturation of a root-canal system with gutta-percha: It lubricates and aids the seating of the master gutta-percha cone, acts as a binding agent between the gutta-percha and the canal wall and fills anatomical spaces that the primary filling material has failed to reach (McMichen *et al.*, 2003). All sealer cements are required to possess certain physical and biological properties (Crane *et al.* 1980 and American National Standards Institute 1984). These properties include biocompatibility, strength, sealing ability, adequate working and setting times, low solubility, and various other characteristics (American National Standards Institute 1984 and Young *et al.*, 1982). Many of these physical properties have been defined in the ANSI specification no. 57 for endodontic sealer cements. Calcium phosphate cements have indicated that they are useful as sealers because they can seal a furcation perforation, are biocompatible and also have potential to promote the healing of bone in endodontic treatment (Cheng *et al.*, 2001)

The time for sealers to set is important clinically. It is desirable to have a setting time that is neither too fast nor too slow. A slow setting time has advantages of enabling the placement of sealer in more than one canal as well as the recovering of gutta-percha from a canal at the time of obturation (if correction is necessary). A

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setting time too slow is a disadvantage as, Coronal leakage may occur soon after the root canal treatment is complete (Hovland and Dumsha, 1985). Possibly a sealer that is unset, or only partially set, may allow more rapid penetration of irritants, such as bacteria or bacterial byproducts, through the obturation. Unset or partially set sealer may permit dislodgment of the apical gutta-percha. Since injectable calcium phosphate cements (iCPCs) possess inferior mechanical strength than the tissue they replace, which restricts them to nonstress-bearing applications (Fukase *et al.* 1990 and Friedman *et al.*, 1998). β -dicalcium silicate was selected for reinforcing the injectable calcium phosphate bone cement in this study.

Since the discovery of calcium phosphate (CPC) by Brown and Chow in 1986, the CPC received much attention over the past 15 years (Markovic *et al.*, 1997 and Miyamoto *et al.*, 1998). A number of CPC variants were developed and their biological affinity was systematically investigated. The current challenge is to place the material in the surgical site by methods as least invasive as possible. It has been showed that using minimally invasive bone cement injection for stabilizing osteoporosis or treating vertebral body fracture has significant clinical potential (Bai *et al.*, 1999). CPC has been developed as an injectable bone substitute material which has many advantages for its good biocompatibility, excellent bioactivity, self-setting characteristic, low setting temperature, adequate stiffness, and easy shaping for any complicated geometry.

Injectable calcium phosphate cements (iCPCs) are used to repair bone defects, but their inferior mechanical strength than the tissue they replace restricts them to nonstress-bearing applications (Fukase *et al.*, 1990 and Friedman *et al.*, 1998). Chow *et al.*, 2000 reported that 65 MPa compressive strength could be obtained by compacting the cement at 700 kPa during setting. Many authors reported the addition of nontoxic salt to the liquid component to improve the strength of the cement. Recently, fibers have been used to reinforce the CPCs (Xu *et al.*, 2000, Zhang and Xu 2005). Polymeric additives, amino salicylic acid, phosphorylated chitosan were also used to improve the mechanical strength of CPC (Miyazaki *et al.*, 1993, Dos Santos *et al.*, 1999). Nevertheless, most of the methods were only suitable for the reinforcement of the preshape type CPC, and not applicable for the reinforcement of the iCPC.

β -Dicalcium silicate was selected for reinforcing the injectable calcium phosphate bone cement in this study. More recently, many authors reported that the silicon-containing bioactive materials, such as bioglass, exhibited excellent bone-like carbonated hydroxyapatite (CHA) forming ability *in vitro* and *in vivo*, and furthermore these materials indicated the potential ability to activate bone-related gene expression and stimulate cell proliferation (Hench *et al.*, 1996, Gough *et al.*, 2004). Especially, β - Ca_2SiO_4 can spontaneously develop strength (spontaneous consolidation) toward water. Some authors have used it as injectable bone cement that showed excellent mechanical properties and bioactivity (Gou *et al.*, 2005). However, β - Ca_2SiO_4 was quite different from the bone mineral of human body, and its hydrated products was essentially non-resorbable *in vivo*.

Calcium aluminate cement is hydraulic binders which do not compete with portland cement in that it gains about 80% of its ultimate strength after only 24 hours from starting the hydration reaction, however, it was considered to be the first with bioactivity properties and which has the necessary mechanical properties to function as a dental material (Loof *et al.*, 2003, L'oe *et al.*, 2005). It has been demonstrated recently that calcium aluminates also have bioactive properties (Ckiewicz *et al.*, 2002, Faris *et al.*, 2006).

Calcium lactate gluconate (CLG) aqueous solution has been evaluated to improve workability, handling properties, antibacterial, biocompatibility and setting time of MTA root canal filling materials as well as root canal sealing materials. CLG solution was prepared by mixing lactic acid, glucono delta lactone, and calcium oxide. The influence of various liquid phases on initial setting time, handling properties, and pH value were investigated. Results showed that by using 23.1wt% CLG solutions as a liquid phase decreased the setting time of WMTA from 155.0 ± 5.0 minutes to 12.3 ± 2.5 minutes and improved the handling properties of MTA to become similar to typical intermediate restorative material. The pH values for hydrated white MTA with deionized water and 23.1wt% CLG solutions were 12.29 ± 0.02 and 11.81 ± 0.04 respectively at 72 hours. X-ray diffraction characterized the crystalline property of the CLG powder as an amorphous powder (Hsieh *et al.*, 2009).

This work aims at investigating of the influence of impregnated calcium lactate gluconate on physical properties and adaptability of new formulated composite composed of β - Ca_2SiO_4 , CAC and biphasic CPC that can be used as a root canal sealing materials.

Materials And Methods

2.1- Synthesis and characterization of the CPC cement:

In order to formulate the calcium CPC based cement, the three basic components were synthesized in the nano size; biphasic compound (composed of 51.7% hydroxylapatite and 48.3% β -tribasic calcium phosphate), calcium aluminate cement and β - dicalcium silicate.

Bi-phasic compound:

The biphasic powder was synthesized from an aqueous solution of $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ and $(\text{NH}_4)_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$ by chemical precipitation method according to the following equation: (S.C. Liou, S.Y. Chen, *Biomaterials* 23 (2002) 4541).



The pH of the reaction was adjusted to 11 by adding ammonia solution gradually and then heating up the reaction medium to 60°C while steering for 1-hour. The deposit was filtered, washed and dried in a drying oven at 110° C for 24- hours and then calcinated at 900 °C for 2- hours.

β-Dicalcium silicate (β-C₂S):

The preparation of β-C₂S (27-30 nano-meter) was achieved by firing molded cubes prepared from a mixture of 2:1 CaO: SiO₂ by weight molar ratio from limestone and quartz (99.6% SiO₂), respectively in the presence of 0.5% boric acid at 1000° C for 2- hours (Ckiewicz *et al.*, 2002). The product was ground, remolded by the aid of carbon tetrachloride and fired at 1450° C for 2-hours. This process was repeated until completion of the reaction as checked by employing the insoluble residue method. The end product was checked for the presence of free lime. The resulting β-C₂S was taken out of the muffle furnace at a temperature of 1000° C and rapidly cooled down to the room temperature. The two synthesized powders components were investigated by X-ray diffraction (XRD) of type (BRUKER axis, D8 ADVANCE, Germany), to verify the identity of the synthesized compound, using a copper target with radiation; wavelength= 1.54-nm, X- ray was generated at 40-KV with a current of 2-5- mA. The scanning speed was 1°/minute. They were also evaluated by Transmission electron microscope (JEM-1230, Joel Co. El. Microscope, Made in Japan) at 100 Kv to evaluate the particle size.

The calcium aluminate cement sample (supplied by Union Alumina Cement - South Korea) used in this work has a chemical composition given in Table (1):

Table 1: Chemical composition of CAC sample.

Constituents	SiO ₂	Al ₂ O ₃	Fe ₂ O ₃	CaO	TiO ₂	Total
CAC	4.88	51.01	1.21	36.32	2.92	96.34

2.2- Synthesis and Characterization of the Calcium Lactate Gluconate powder (CLG):

CLG with the chemical formula of $\text{Ca}_5(\text{C}_3\text{H}_5\text{O}_3)_6(\text{C}_6\text{H}_{11}\text{O}_7)_4 \cdot 2\text{H}_2\text{O}$ by loading 13.5 gm of lactic acid (molecular weight [MW]=90.1 g/mol), 17.8 gm glucono delta lactone (MW=178 gm/mol) and 7 gm of calcium oxide (MW=56 gm/mol) in a container containing 50 ml of deionized water. Contents were stirred over an electric heater until the ingredients were completely dissolved. The material was then filtered and left in a drying oven at 110°C for 3 days until it dehydrated and gave a yellowish crystalline structure. The formed crystals were pulverized in a porcelain mortar to very fine particles (Hsieh *et al.*, 2009).

2.3. - Characterization of CLG powder particles was carried out by using:

- A. Fourier Transform Infra Red Spectrometer (FTIR) [FTIR 4100, JSCO, Japan] Mass Spectrometer
- B. Nuclear Magnetic Resonance Spectrometer (NMR) [Oxford - NMR 300, USA]
- C. (MS) [Joel DMS DX303 mass spectrometer, USA.]

2.4. - Preparation of the mixing liquid:

CLG solutions of different concentrations; 17 wt%, 19 wt%, 21 wt%, 23 wt%, 25 wt%, 27 wt%, were prepared to be used as a mixing liquid to prepare the sealer paste by using a certain liquid to solid ratio that will be given later.

Table 2: The composition of the experimental powder:

Constituents	mix composition Weight %
Bi-phasic compound composed of: 51.7% hydroxylapatite and 48.3% β - tribasic calcium phosphate. *	30
Calcium aluminate (CAC)	20
β -dicalcium silicate.	45
Bismuth oxide.	5

2.5- Adjusted proportions of experimental powder to liquid ratio:

It was found that to reach the acceptable working consistency of the cement one well packed and flushed scoop of weight equals to 0.1068 gm of the powder, was mixed with one-drop weight equals to 0.0227 of the liquid. The spatulation was carried out on a clean smooth glass slab at $37^{\circ}\text{C} \pm 1$, using a rigid stainless steel spatula in a circular motion while pressing the spatula hard to incorporate the powder into the liquid. The spatulation of the powder incorporated into the liquid phase was done as successive fractions, one six of the powder was added every 15 seconds and kneaded with a spatula between addition to produce a paste with a workable consistency. The cement exhibited a string of one inch as the spatula was raised slowly from the glass slab.

2.6- Physical characteristics of the experimental sealer:

The sealer was subjected to setting, working time and dimensional stability tests, which were, carried out according to the ANSI specification no. 57 (American National Standards Institute, 1998) for root canal sealers and cements.

2.7-pH, calcium ion release and adaptation evaluation:

15 freshly extracted human lower first premolars were collected. Teeth were thoroughly washed under running water to clear away any remnants of blood and debris. They were then immersed in 5.25% sodium hypochlorite (NaOCl) for one hour, then the solution was changed and they were placed in a new one for 24 hours to remove the soft tissue remnants from the root surface. Crowns of the teeth decapitated at a fixed distance from the apical end 17-mm with a high-speed fissure bur under water spray. Teeth were then stored in saline till time of use.

Root canal was prepared with modified crown down preparation with series of files up to size 35 with REVO-S rotary system. Root canal was frequently irrigated with 10-ml of 17% EDTA and 15-ml 5.25% NaOCl between instrumentation and a final rinse was performed with 5- ml NaOCl. File was discarded after the preparation of six canals or if any sign of strain was evident. Paper points were used to dry the canals thoroughly to be ready for the obturation. Canals were obturated using a single Micro- Mega Gutta-percha size #35 pre-fitted to the working length. The roots were kept in moist gauze during instrumentation and subsequent filling. Cement was placed in the canal by repeated coating and insertion with the master cone. Finally, the pre-fitted master cone was then coated with sealer and introduced apically into the canal. The cone was subsequently seared off with a hot plugger. The occlusal aspect of each root was sealed with Glass Ionomer. The teeth were randomly divided into three groups of five teeth each according to the time of immersion in distilled water; one week, two weeks and one month.

Each tooth was immersed in a plastic container, containing 10-ml of distilled water, kept at a constant temperature of 37°C and at 100% relative humidity. Filled teeth (n=5) were removed at the selected immersion time and air-dried. The pH of the distilled water in which filled roots were immersed for the three immersion periods was measured. In addition, the distilled water was analyzed by AAS for Ca^{++} ion release measurements. The roots were placed in acrylic molds 2x2- cm to facilitate their handling. Roots were transversely sectioned under water spray at 2- mm from the anatomical root apex to resemble an apical section. Then the selected sections were mounted on aluminum stubs, identified, placed in desiccators, and then coated with a 20-nm palladium-gold film to be ready for Scanning Electron Microscope examination at X-1000 magnification.

Results And Discussion

Analysis of particle size of synthesized components using Transmission Electron Microscope:

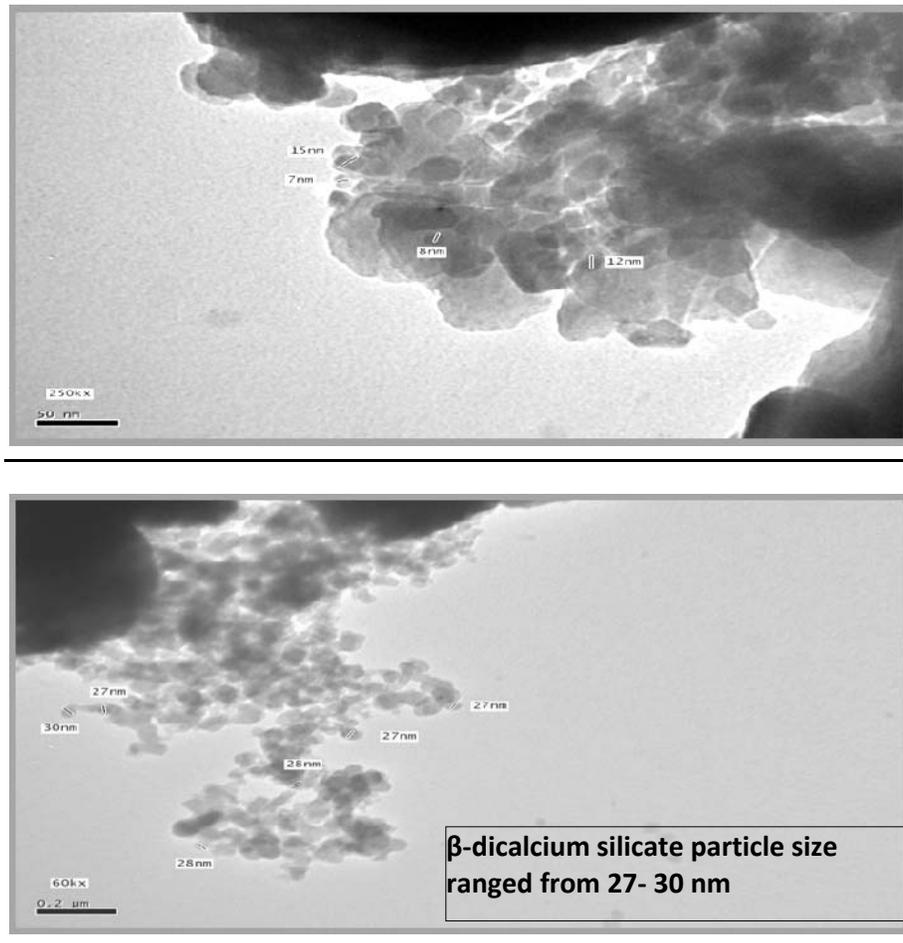


Fig. 1: transmission electron photomicrograph showing that the bi-phasic and the di-calcium silicate compounds are in the nano-size. Nano-sized particles varied in the three experimentally synthesized materials.

Characterization of the synthesized CLG powder:

The chemical structure of CLG that has the chemical formula of $\text{Ca}_3(\text{C}_3\text{H}_5\text{O}_3)_6(\text{C}_6\text{H}_{11}\text{O}_7) \cdot \text{H}_2\text{O}$ is shown in fig.(4). The results of mass specimen confirmed that the molecular weight of calcium lactate gluconate (CLG) compound which is equal to 324.295. Nuclear magnetic resonance (NMR) given in fig. (3) was also used to confirm the chemical structure for CLG as it revealed the presence of CH_3 , CH_2 , H_2O and OH groups. The presence of O-H , C=O , C-H , CaCO_3 and Ca(OH)_2 has been detected with the aid of IR analysis as shown in fig. (2).

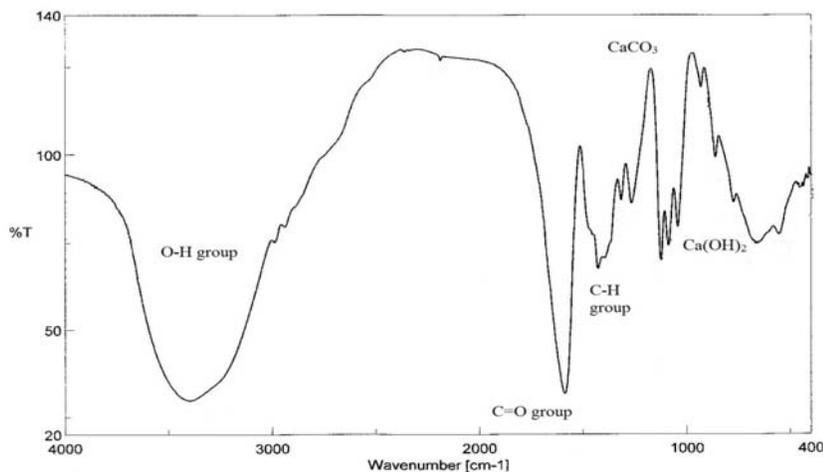


Fig. 2: FTIR chart of a sample of CLG powder

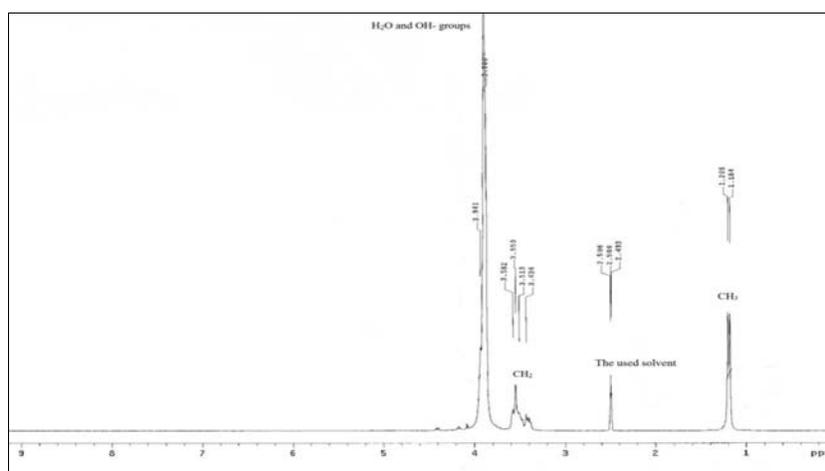


Fig. 3: The NMR of a sample of CLG powder

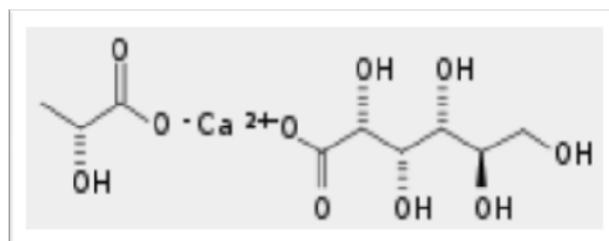


Fig. 4: The chemical structure of CLG. The M.wt. = 324

The concentration range of CLG solutions used in preparing the pastes was 17, 19, 21, 23, 25 and 27 wt percent. The different CLG solutions were prepared with deionized water and it was stated that this range of concentration possesses an excellent workability and acceptable setting times (Hsieh *et al.*, 2009), which are considered essential for the suitable evaluation of the material to be used in the dental field.

Workability and setting times:

The influence of using different concentrations of CLG solution on both workability and setting times is shown in fig. (5) and (6) In a pilot study, the effect of CLG solution on setting time of the cement mix was investigated starting from very low concentration and up to extremely high weight percent (wt%) of CLG solution. The study reveals that there is a concentration range of CLG solutions, which showed considerable setting and workability times, that is from 17 wt% to 27 wt%. The results showed that when using CLG solution of concentration below 17% and above 27% to prepare the cement paste the setting time is very long and the working time becomes short. Fig. (5) showed that the setting time decreases with the increase of CLG solution up to 21% and then starts to increase again from 23% and up to 27%. On the other hand, fig. (6), which represents the workability time against CLG solution concentration, showed the reverse effect of the setting times. This means that the concentration range of CLG solution that give the shortest setting time and the longest workability time will be considered for mixing of the synthesized powder for dental applications.²⁹ Depending upon the results of the pilot study, the use of CLG solution in a concentration range between 21 to 23 wt% can form a biocement paste of an excellent workability and minimum setting times of about 8.5 and 17 minutes respectively. The concentration used for obturating the teeth in this study was 23%. This percentage provided the highest workability time (8.5) which gives the dentist time for manipulation of the material, and a low setting time (20 min), which allows for fast curing of the material, which in turn decreases the possibility of fluids leakage into the canals.

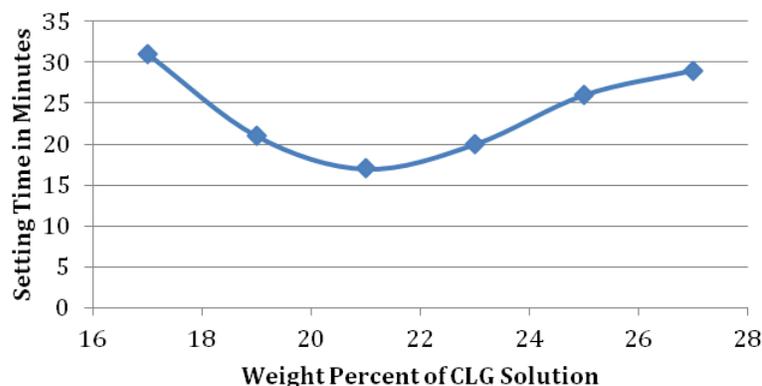


Fig. 5: Setting time as Influenced by addition of different amounts of CLG to the mixing liquid.

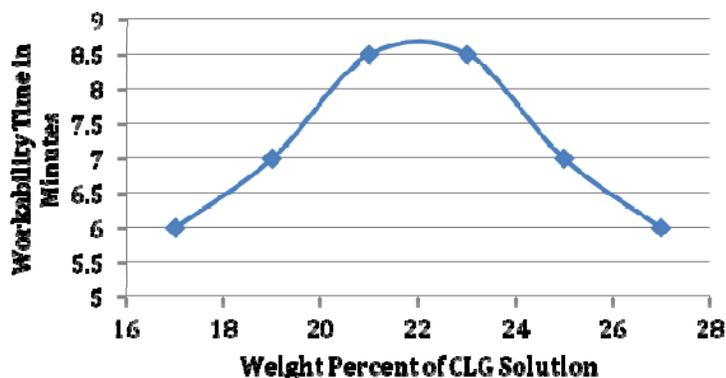


Fig. 6: Workability time as Influenced by addition of different amounts of CLG to the mixing liquid.

Dimensional stability:

Endodontic sealers play an important role in preventing leakage of root canal fillings, without which, the canals would leak (Kontakiotis *et al.*, 1997; Wu *et al.*, 2000). Complete obturation of the root canal system with a dimensionally stable material is a major goal in conventional root canal therapy (Silver *et al.*, 1999), since dimensional change affects the integrity of the bond between the sealer and the root dentin or core material (Kazemi *et al.*, 1993).

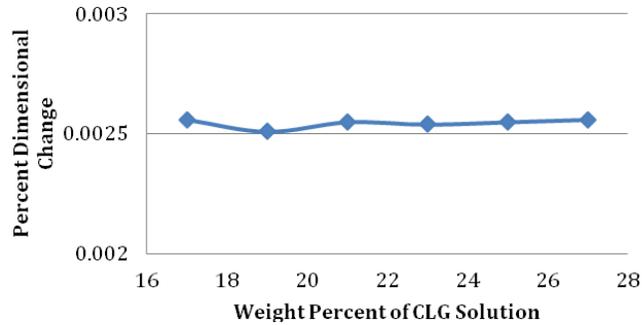


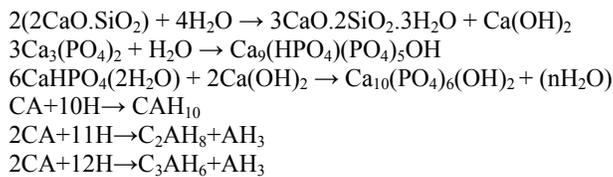
Fig. 7: Influence of different amounts of CLG in mixing liquid on percent dimensional stability.

All the concentrations used in this study exhibited expansion. The swelling of the calcium phosphate cement could be attributed to the formation of calcium oxide (CaO); this comes in agreement with Serraj *et al.*, 2002. Further researches should be directed to clarify the cause and effect of CaO.

Fig. (7) represents the percent dimensional change of the pastes prepared from the synthesized biocement using the recommended range of concentration of CLG mixing solution. It is clear from the figure that the influence of the different concentrations of CLG solution is very little at concentration of 19% there is a little degree of expansion, which by increasing the concentration of CLG up to 27% increases.

pH and Calcium ion release:

There is a little rise of pH through the test periods; 7.78 after one-week immersion, 8.1 after two weeks immersion and 8.18 after one month immersion, which are represented in fig. (8) that is due to the hydration of calcium silicate. This small amounts of Ca(OH)₂ maybe consumed in the rest of hydration reaction of phosphate and aluminate cements leading to a decrease in calcium ion concentration throughout the hydration period as shown in fig. (9). This means that the dissolution of the hydrated paste decreases with the increase of the pH of the hydration medium as shown in fig. (10) the hydration equation of each compound present in the biocement composite may discuss the above mentioned behavior as shown in the following chemical equations:-



The large amount of released calcium ions coincides with the results of Ghazvini *et al.*, 2009, who stated that the release of Ca(OH)₂ from the hydration reaction of dicalcium silicate leads to a rise in pH and calcium ion released. Serraj *et al.*, 2002, also stated that; "the di- basic calcium phosphate - hydroxyl apatite network acts as a drug delivery system, releasing hydroxyl ions"

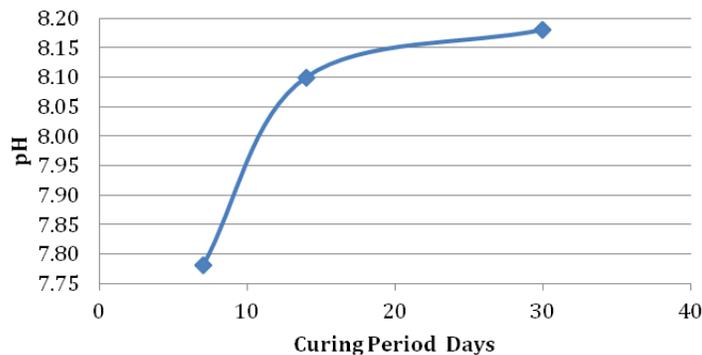


Fig. 8: Influence of curing periods on the pH of the immersing distilled water.

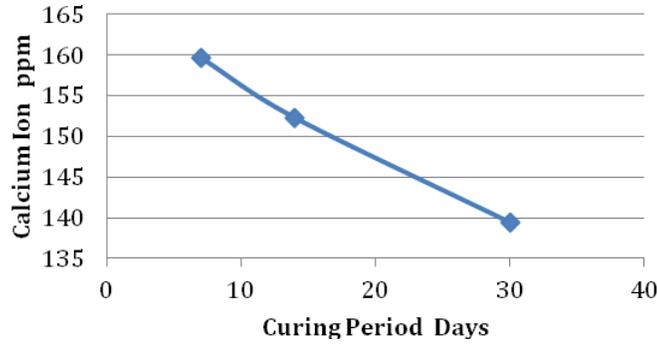


Fig. 9: Influence of curing periods on the calcium ion concentration of the immersing distilled water.

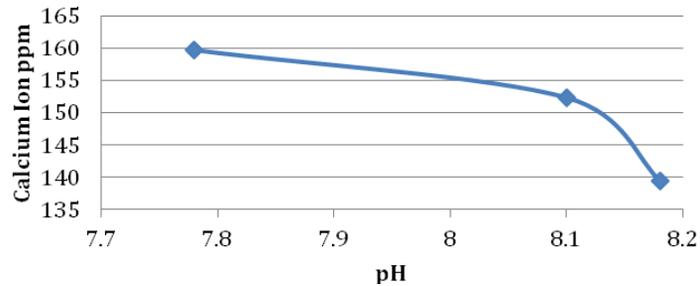


Fig. 10: Relationship between calcium ion concentration and the pH of the immersing distilled water.

FTIR Analysis:

The FTIR spectra of the hydrated samples prepared with CLG mixing liquids of different concentrations together with the blank sample mixed with distilled water, (S1=blank, S2=17% CLG, S3= 19% CLG, S4= 21% CLG, S5= 23%CLG, S6= 25% CLG, S7= 27% CLG) are given in Fig. (11) and illustrated in Table (3). The FTIR spectra showed the appearance of all the expected bands (Table. 3), namely, the apatite bands at 1030, 600 and 560 cm^{-1} , the aluminate bands at 590, 665 and 945 cm^{-1} , the molecular water incorporated with the hydrated phases at 1645 and 3365 cm^{-1} , carbonated apatite at 1490, 1460, 1420 and 1410 cm^{-1} and bands corresponding to CSH resulted from the hydration of $\beta\text{-C}_2\text{S}$ at 445 and 950 cm^{-1} (Maria *et al.*, 2011). The carbonated apatite is formed due to the possible carbonation with the atmospheric CO_2 . The increasing trend of almost all of the IR bands mentioned above with the increase of CLG percentage in the mixing liquid can be attributed to its accelerating effect of the hydration reaction of the synthesized bio-composite cement with distilled water. This will enhance formation of more hydration phases more than the blank sample, which is mixed without the CLG solution.

Table 3: Results of FTIR analysis.

Wave number cm^{-1}	Vibrational mode	Crystal Phase
540 s,b 590 sh 665 m	Aluminate coupling vibrations	Calcium aluminate cement and the hydrated aluminate phases
805 vw 945 sh	Aluminate	
1635 sh 1645 m-w	$\delta_2 \text{H}_2\text{O}$	The molecular water incorporated in the formation of hydrated compounds
3365 s	$\delta_2 \text{H}_2\text{O}$	
3620 s 3670 s	δHO δHO	The hydroxyl group found in both hydrated phases and in the chemical formula of CLG
1030 b 962-960 sh	$\delta_3 \text{PO}_4^{3-}$ $\delta_1 \text{PO}_4^{3-}$	Antisymmetric stretching Amorphous calcium phosphate Apatite.
600 – 560sh 565 b	$\gamma_4 \text{PO}_4^{3-}$ $\delta_4 \text{PO}_4^{3-}$	Amorphous calcium phosphate B-type carbonated apatite. Amorphous calcium phosphate
1490-1460 m 1420-1410 m	$\delta_3 \text{CO}_3^{2-}$ $\delta_3 \text{CO}_3^{2-}$	Antisymmetric stretching B-type carbonated apatite.
445 vw 950 sh	CSH phase	The phase formed due to the hydration of the belite phase ($\beta\text{-C}_2\text{S}$)

vw=very weak w=weak m=medium s=strong b=broad sh=shoulder

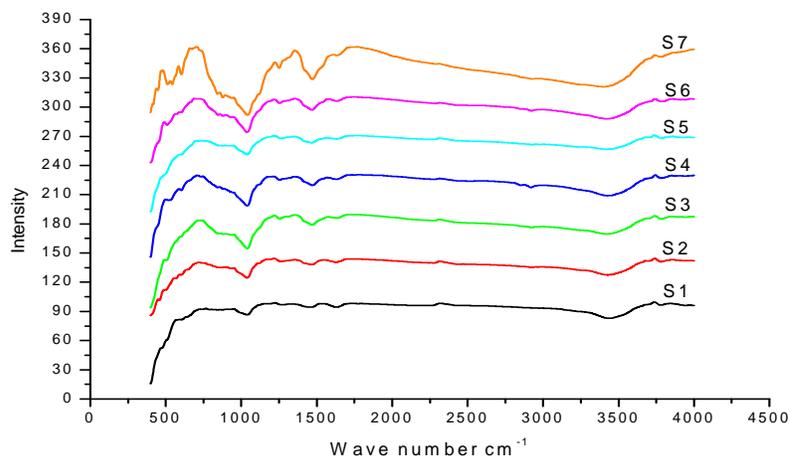
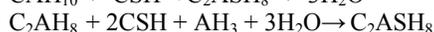
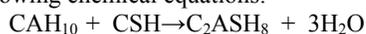


Fig. 11: IR spectra of pastes cured for 7 days and prepared with distilled water and with different CLG solutions.

XRD analysis.:

The influence of different concentrations of CLG mixing liquid on the hydration behaviour of the synthesized bio-cement was studied by XRD-analysis. The blank bio-cement that is mixed with distilled water and those mixed with the different concentrations after same hydration period of 7 days (S1=blank, S2=17% CLG, S3= 19% CLG, S4= 21% CLG, S5= 23%CLG, S6= 25% CLG, S7= 27% CLG) are represented in Figure (12). As was mentioned above, the CLG mixing solution plays its role only during the very early hydration (setting time) but it has a very little effect on the later curing period. Both β -C₂S and the biphasic compound hydrates with water in a very slow rate, so, the use of CLG solutions was very important in order to enhance and accelerate the hydration rate of those phases. The synthesized composite contains 20% of calcium aluminate cement (CAC), which completes 80% of its hydration process during the first 24hrs giving the main hydration phases (CAH₁₀, C₂AH₈ and C₃AH₆) as was mentioned above in the chemical equations. The hydrates CAH₁₀ and C₂AH₈ are hexagonal and they are metastable however, they converted at elevated curing temperature of above 30°C to the more stable cubic compound C₃AH₆ and AH₃, through a chemical process cold the conversion process (Taylor 1990). The conversion process results in considerable changes in the morphology, mineral nature and physico-mechanical properties of the cement pastes. However, in our case this conversion process may be compensated by the reaction of the 2 main phases, i.e., CAH₁₀ and C₂AH₈ with the main hydration phase of β -C₂S to form the more stable Stratling's compound (C₂ASH₈) according to the following chemical equations:



Investigation of the XRD patterns given in Fig. (12) reveals that there is a slight difference in peak height of most of the anhydrous and hydrated phases for the blank sample that is mixed with distilled water and those are mixed with the CLG solutions. The use of CLG solutions with different concentrations enhances the hydration of both calcium silicate and the biphasic compound that are hydrated slowly with water which is responsible for prolonged setting times. This could be detected in Fig.(12) by the decreasing trend of the anhydrous peak from the blank sample (S1) to those mixed with CLG solutions (S2, S3,S4,S5,S6 and S7). As can be seen from peak identification on the XRD patterns, an overlapping of the peaks characteristic for the anhydrous and hydrated phases makes the height of some peaks to be enhanced due to precipitation of such hydration compounds. The absence of the characteristic XRD peak (at $2\theta=18^\circ$) of Ca(OH)₂ (portlandite) that is release as a by-product from hydration of β -C₂S could be attributed to slow rate at which the β -C₂S hydrates and to its reaction with CLG to form the calcium salts of lactic and gluconic acids.

Prepared roots were obtured with the tested cements using the single cone technique that coincide with the prepared canal (Schäfer and Zandbiglari 2003). In the present study the volume of the cement was uniform and continuous around the gutta-percha cone. The volume of the sealer used in the single-cone technique was minimized because gutta-percha cones were calibrated to the preparation. A transverse section to represent the adaptation in the apical third of the filled canal was examined by SEM. The experimental sealer showed good adaptation with dentin, which is possibly due to its small particle sizes that enable them to penetrate into the open dentinal tubules along the canal wall; as previously suggested by Krell and Wefel 1984. Results of the scanning electron microscope after one-week immersion in distilled water showed good adaptation of the sealer

to both the tooth and the gutta-percha, whereas after two weeks immersion, a slight separation at the gutta percha-sealer interface was evident. After one-month immersion in distilled water, more separation of the sealer from the gutta-percha took place, this may be attributed to dissolution of the sealer due to the prolonged immersion period in distilled water.

On the other hand, adaptation could be due to calcium silicates, which do not shrink during setting (Krell and Wefel 1984, Schäfer and Zandbiglari 2003).

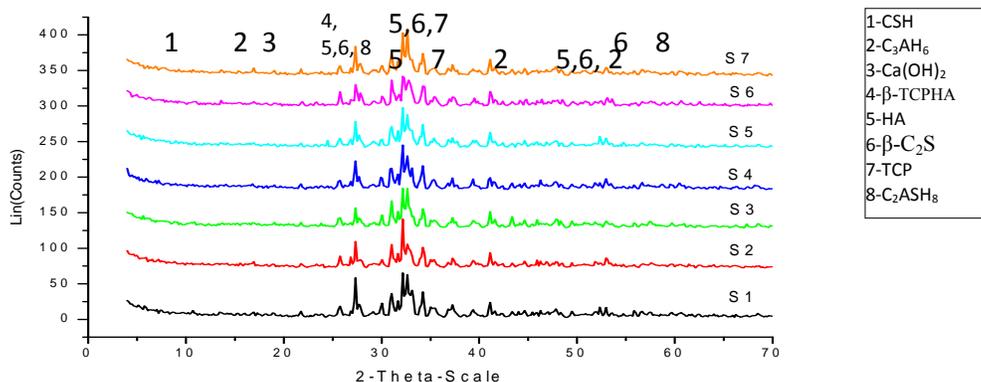
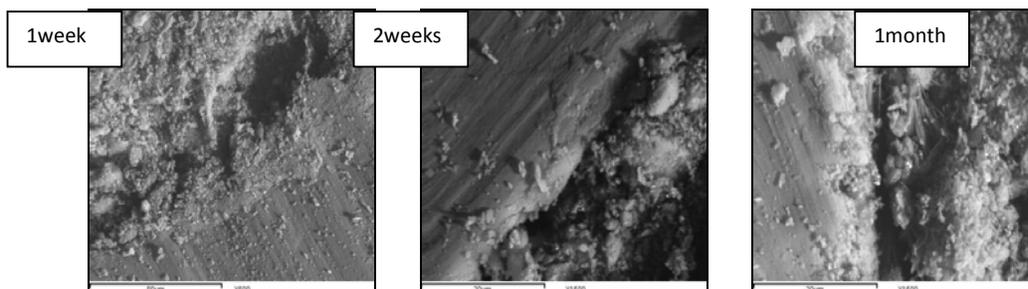


Fig. 12: XRD Patterns of pastes cured for 7 days and prepared with distilled water and with different CLG solutions.

Adaptation:



Conclusion:

According to the findings the following conclusions are drawn:

1. Nano-particle size did influence physical characteristics of the experimental calcium phosphate cements, improved chemical reactivity, biocompatibility and decreased setting times.
2. Using of mixing liquids containing controlled amounts of CLG did enhances the hydration reaction of main constituents of the synthesized bio-cement.
3. Preparation of bio-cement pastes with mixing liquids of different CLG weight percentages didn't have an adverse effect on dimensional stability.
4. CLG solutions of concentration ranges from 19 to 23 wt% showed an excellent workability and very good range of setting time that are acceptable in dental application.
5. Experimental sealers showed expansion that resulted in gap free interface with the tooth.
6. The experimental sealers showed a rise in high pH and calcium ion release.

Recommendations:

1. A future study of slicing the filled roots with cone beam computed tomography (CBCT) could define the existence of gap free interface along full length of the filled canal.
2. Smoothness and uniformity of the synthesized cements and the penetration of the dentinal tubules encourages their use in clinical cases; provided that biological and cytotoxicity studies should be considered first.

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