Research Journal of Pharmaceutical, Biological and Chemical Sciences

Formulation And Characterization Of A Calcium Silicate/Calcium Phosphate Root End Filling Material; Part I: Synthesis And Physico-Mechanical Properties.

MM Radwan[1], Engy M Kataia[2], and Maram E Khallaf[2]*.

[1]Ceramics department, National Research Centre, Dokki- Giza - Egypt.
[2]Restorative and Dental Materials Departement, National Research Centre, Dokki- Giza - Egypt.

ABSTRACT

Tricalcium-silicate and Tetracalcium-phosphate were prepared from ultra-pure chemical compounds via solid-state reaction at 1450°C and 1500°C respectively in an attempt to formulate a root end filling cement. Materials were investigated by X-ray diffraction and Transmission-electron microscope. The formulated cement comprises 60 wt% Tricalcium-silicate, 20 wt% Tetracalcium-phosphate and 20 wt% Bisthmus oxide. Setting-time, micro-hardness, dimensional-stability, calcium ion release, and radiopacity were examined. The desired setting time was adjusted using mixing liquids of different Calcium Chloride concentrations. The cement expanded by 0.17%. Regarding radiopacity, the formulated cement had significantly higher values than MTA. The material continuously released Calcium ions throughout the testing period. MTA showed significantly higher microhardness than the experimental cement at 7 days. Finally, the prepared composite showed good physical and mechanical properties. Further leakage, adaptation and biocompatibility studies will be conducted in part II.

Keywords: Calcium Chloride, Calcium Phosphate, Calcium Silicate, microhardness, physical properties.
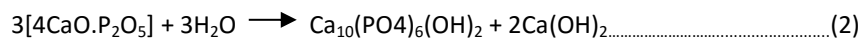
*Corresponding author

INTRODUCTION

Periradicular surgeries aim to remove the cause of the disease thus providing a favorable environment for healing. Root end filling placement is an important step in managing root end[1]. Several studies have demonstrated the excellent biocompatibility, osteogenic[2], and physicochemical properties of Mineral Trioxide Aggregate (MTA)[3]. MTA is composed of four main phases; C_3S, C_2S, C_3A and C_4AF in addition to Bi_2O_3 as radiopacifier with no effect in setting and hardening processes[4]. Calcium-silicate, which is the main constituent of MTA undergoes a hydration reaction when mixed and stored in distilled water resulting in an insoluble final set hardened structure of high hydraulic properties[2]. Although MTA has good mechanical properties, it presents some undesirable characteristics such as; sandy consistency, poor dispersion, high porosity[5], long setting time, high cost, difficult manipulation and insertion[6]. Therefore, changes in formulation/composition and development of new branches in this class of materials in attempts to improve its physicochemical characteristics and evaluation of their properties is essential[7]. Tricalcium-silicate (C_3S), which is responsible for the superior physicomechanical properties of MTA has been recently used in root-end filling materials[8]. To avoid any contamination from trace elements like those found in MTA, C_3S was prepared using ultra pure chemical materials[9]. Increasing interest has been assigned to calcium-phosphate bio-ceramics, namely; hydroxyapatite $Ca_{10}(PO_4)_6(OH)_2$(HA), Tetracalcium-phosphate ($Ca_4(PO_4)_2O$, Tricalcium-phosphate $Ca_3(PO_4)_2$(TCP) and biphasic calcium-phosphates (HA-TCP mixture), to be used in medical applications[10]. Among these materials, Tetracalcium-phosphate (TTCP), has been used in dental applications as root canal sealers[11]. TTCP that has a stoichiometric Calcium-phosphate ratio greater than hydroxyapatite (HA) and synthesized via solid state reaction at elevated temperature (above $1450^{o}C$), is a metastable compound and needs rapid cooling stored in the absence of moisture to avoid decomposition to free lime and HA[12]. TTCP has the highest solubility among all calcium-phosphate bio-ceramics and undergoes hydration reaction like C_3S phase when mixed with distilled water.

The hydration of C_3S is shown in Eq. (1)[13].

$$3CaO.SiO_2 + \sim 5.3H_2O \longrightarrow \sim 1.7CaO.SiO_2.4H_2O + \sim 1.3Ca(OH)_2 \quad \text{................(1)}$$
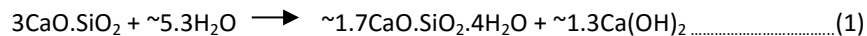
The hydration reaction of TTCP is given in Eq. (2):

$$3[4CaO.P_2O_5] + 3H_2O \longrightarrow Ca_{10}(PO4)_6(OH)_2 + 2Ca(OH)_2 \quad \text{................(2)}$$

The reaction products are stoichiometric HA and calcium-hydroxide[14]. Thus, the aim of this work is to formulate an advanced nano-sized bioceramic composite prepared from calcium-silicate and calcium-phosphate with the addititon of bismuth-oxide as a radio-opacifier to be used as a root end filling and to investigate some of its physical and mechanical properties.

MATERIALS AND METHODS

Materials preparation and characterization

Powder components

C_3S phase (13-nanometer) was synthesized by firing molded cubes of 3:1 $CaO:SiO_2$ molar ratio, using ultra pure limestone and quartz (99.6% SiO_2), in the presence of 0.5% boric acid at 1000ºC for 2 hours[15]. The product was ground, remolded using carbon tetrachloride and fired at 1450ºC for 2 hours. The firing process was repeated till the reaction was completed. The end product was checked for the presence of free lime.

The most common method used for preparing TTCP is limited to solid-state reaction at high temperature[16]. This method is usually based on mixtures of calcium carbonate ($CaCO_3$) and monocalcium-phosphate-monohydrate ($Ca(H_2PO_4).H_2O$) in a molar ratio of 3:1 in n-heptane for 16-hours. The slurry was filtered, dried at room temperature and heated to 1450-1500°C for 6-12h and rapidly quenched to room temperature according to the following chemical reaction:

$$3CaCO_3 + Ca(H_2PO_4)_2.H_2O \longrightarrow Ca_4(PO_4)_2O + 3CO_2 + 3H_2O$$
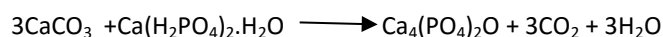
The resulting powders were milled in n-heptane for 8h, dried at 100°C, and kept under vacuum to avoid hydration[17].

Mixing Liquid

The mixing liquids were prepared using chemically pure calcium-chloride ($CaCl_2$). Four different calcium-chloride solutions of concentrations; 2, 4, 6 and 8wt% has been used in order to determine the most suitable setting time for the investigated cement to be used as a root end filling material.

X-ray diffraction (XRD)

XRD was used to verify the identity of the two synthesized compounds, using a copper target with radiation; wavelength=1.54nm, X-ray was generated at 40-KV with a current of 2-5Ma.

Transmission Electron Microscope (TEM)

The final materials were finely ground in agate mill to the desired particle size and evaluated by TEM, scanning speed was 1°/minute using TEM (JEM-1230) at 100Kv.

Physical characteristics of the experimental cement (CSP)

CSP was mixed in powder/liquid ratio 3:2. ***Setting time*** and ***dimensional stability*** were measured three times according to the ANSI/ADA specification no.57 (American National Standards Institute/American Dental Association) for root canal cements[18]. The mean values and standard deviation were calculated and recorded.

Radiopacity

CSP and Mineral Trioxide Aggregate (MTA) were mixed and packed into a stainless steel ring mold according to the International Standard ISO 6876 [19]. Six specimens were prepared for each material. Specimens with no visible defects were positioned on photo-stimulable phosphor plate (Soredex, Digora, Helsinki, Finland) with the Aluminium step wedge of thickness 2-16mm in 2mm increments. A dental x-ray system (Progeny, Midmark, USA) operating at 60kV, 10mA for 0.3 second exposure time with a 30cm focus-film distance was used. Xray images were analysed using the Digora 1.51 software (Orion Corporation Soredex, Helsinki, Finland). Equal-density areas in the radiographs were identified by the equal-density tool to compare the radiographic density of the cements and the radiopacity of the different aluminium stepwedge thicknesses. Three readings were taken for each specimen and their average was calculated. The radiopacity value was determined according to the following equation as described by the following equation [20]:

$$\frac{AX2}{B} + mmAl \text{ immediately below RDM}$$

where:
A=radiographic density of the material (RDM) – radiographic density of the aluminium stepwedge increment immediately below RDM.
B=radiographic density of the aluminium stepwedge increment immediately above the RDM – radiographic density of the aluminium stepwedge increment immediately below the RDM.
2=2 mm increments of the aluminium stepwedge.

Calcium ion (Ca^{++}) release

Fourteen extracted, human single-rooted teeth were chosen. Teeth were decoronated at cementoenamel junction. A #15 K-file was used to measure the working length until it exited from the apical foramen. Preparation of the root canals was done using Protaper universal files (Dentsply, Maillefer, Switzerland) till size F3 with 2% NaOCl as an irrigant between each file. Root canals were obturated with Protaper gutta-percha size F3 and endofill sealer (Dentsply, Switzerland). Orifice was sealed with a temporary filling (Cavit, 3M ESPE, United States) to prevent leakage. Roots were kept in deionized water for 1-week at

room temperature to ensure complete setting of the sealer. 3-4mm were resected apically perpendicular to the long access of the root using fissure bur in a high-speed handpiece with water coolant. Apical cavity of depth 3 mm was prepared using #1 round bur in a high-speed handpiece with water coolant.

Roots were divided into 2 groups according to incubation period (1-week and 1-month). CSP was mixed in powder/liquid ratio 3:2. CSP was condensed into the apical cavity and moist cotton was placed around root apex. Each root was placed in a plastic container, containing 10-ml of distilled water, kept at $37^{o}C$ and 100% relative humidity. After 1-week, seven roots were removed and the Ca^{++} released in the water where they were immersed was analyzed by atomic absorption spectroscopy (AAS). 1-month later, procedure was repeated with remaining 7 molars. The mean values and standard deviation were calculated and recorded.

Microhardness

MTA and CSP were mixed and condensed with an amalgam carrier and hand condensers into 14 acrylic cylinders with internal diameter and height of 3-mm. Condensation was done against a glass slab. The surface facing glass slab was marked. To ensure complete setting, specimens were kept in 100% humidity at 37ºC for 24-hours. The unmarked surface of each sample was wet-polished at room temperature using sandpaper discs (600-grit and 1,200-grit, 3M, St Paul, MN) and minimum hand pressure.

Vickers microhardness tester (Nexsus 4000/60, INNOVATEST, Netherlands, Europe) in Vickers Hardness Units (VHN) was used with a load of 500g and dwell time of 5-sec. The microhardness of the unmarked surface was examined for each sample. The resulting indentation was measured under a microscope and VHN was calculated. Three separate indentations were made for each surface at least 1mm apart. The mean of the 3 readings was taken as the microhardness of that sample.

RESULTS

XRD:

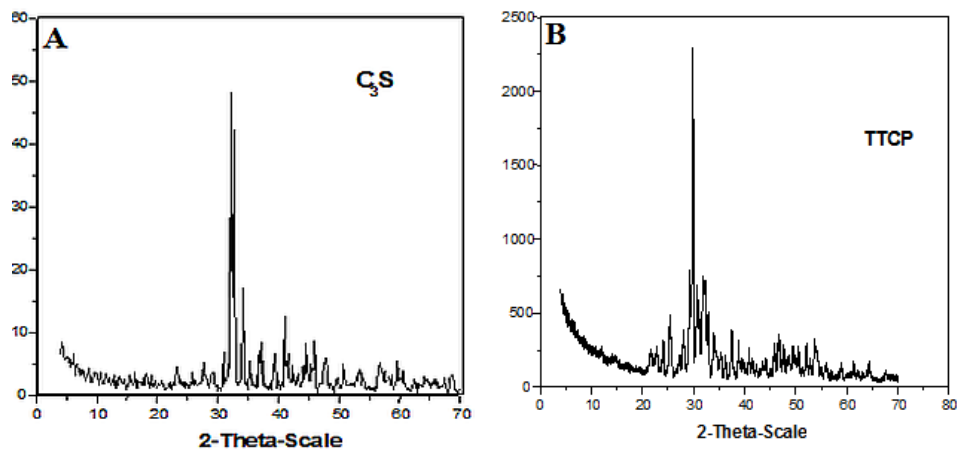Results of XRD are shown in Fig. 1.

Fig. 1: XRD spectral analysis (2-Theta-scale) of the prepared phases (A) C₃S - (B) TTCP.

TEM

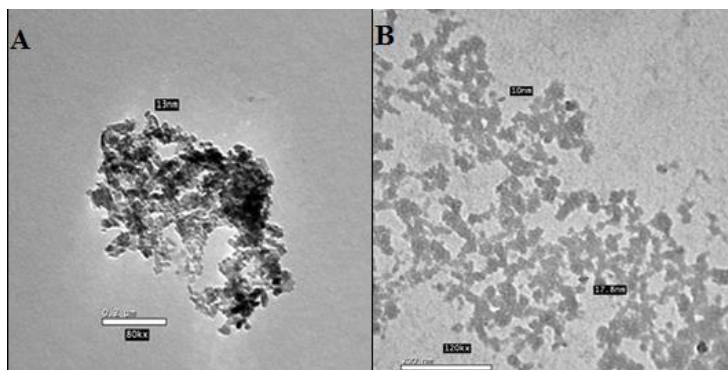The desired particle size was reached as shown by TEM (Fig.2).

Fig. 2: Transmission electron photomicrograph of both synthesized materials. (A) C3S particle size ranged from 13nm, (B) TTCP particle size ranged from 10-17nm.

Physical properties

Setting time

As mentioned before, the prepared bioceramic composed of 60% C_3S with a relatively longer setting time compared to that of calcium-phosphate cement, so, the setting time of the synthesized biocomposite was carried out using different concentrations (0, 2, 4, 6 and 8 wt%) of $CaCl_2$ solutions in order to get the desired setting time needed for use as a root end filling. The $CaCl_2$ salt had an accelerating effect on the hydration reaction of the cement pastes, which shortens the setting time as shown in Fig.3. As shown in the setting time curve, the use of 2 and 4% $CaCl_2$ mixing liquids showed rapid decrease in setting time than the 6 and 8% solutions. The 4% $CaCl_2$ solution that shows 4-min setting time was taken as the optimum mixing liquid for this investigation.
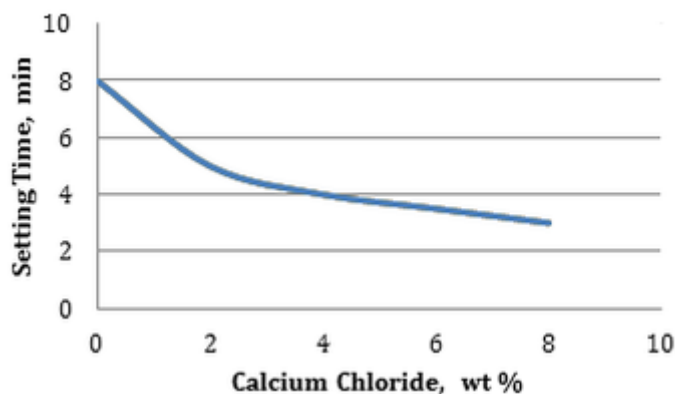

Fig. 3: Influence of concentration of calcium-chloride solution on setting time of the synthesized composite.

Dimensional stability

The tested cement mixed with both distilled water and 4wt% $CaCl_2$ showed expansion. When mixed with distilled water expansion was 0.16% and when mixed with 4wt% $CaCl_2$ it expanded by 0.17%.

Radiopacity

Radiopacity values were statistically significantly higher for CSP (7.95 ± 0.68) than MTA (3.71 ± 0.69) where (p=0.001) (Table 1).

Table (1): The mean, standard deviation (SD) values of RadiOpacity values (mmAL).

Variables	Mean ± SD
CSP	7.95 ± 0.68
MTA	3.71 ± 0.69
P-value	0.001*

CSP= Experimental Calcium silicate/phosphate cement.
MTA= Mineral Trioxide Aggregate
*; significant (p<0.05)

Calcium ion Release

CSP showed Ca^{++} release after one week immersion of (513.49 ± 71.34) which increased but not statistically significant after one month immersion (680.69 ± 53.34) where (p=0.006). (Fig.4).
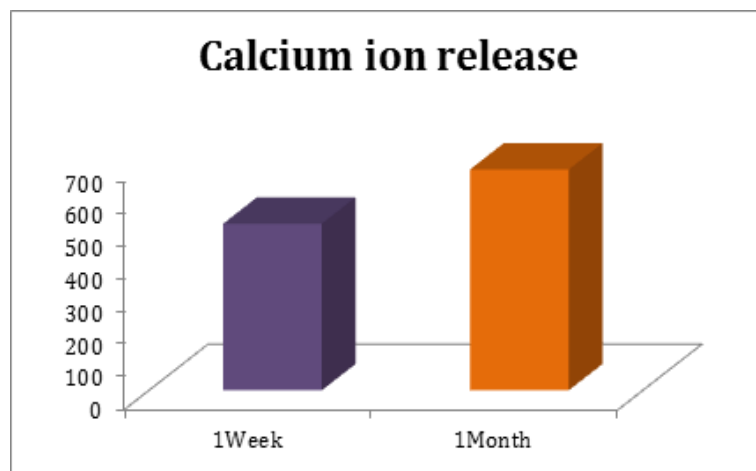

Fig. 4: Bar chart representing Calcium ion release at 1 week and 1 month.

Microhardness

Microhardness results for both materials are shown in Table 2. For both CSP and MTA, no statistically significant difference was found between 24hrs and 7days. Also no statistically significant difference was found between (CSP/24hrs) and (MTA/24hrs). While a statistically significant difference was found between (CSP/7days) (40.24 ± 13.84) and (MTA/7days) (63.26 ± 15.93), where (p=0.04) (Fig.5).
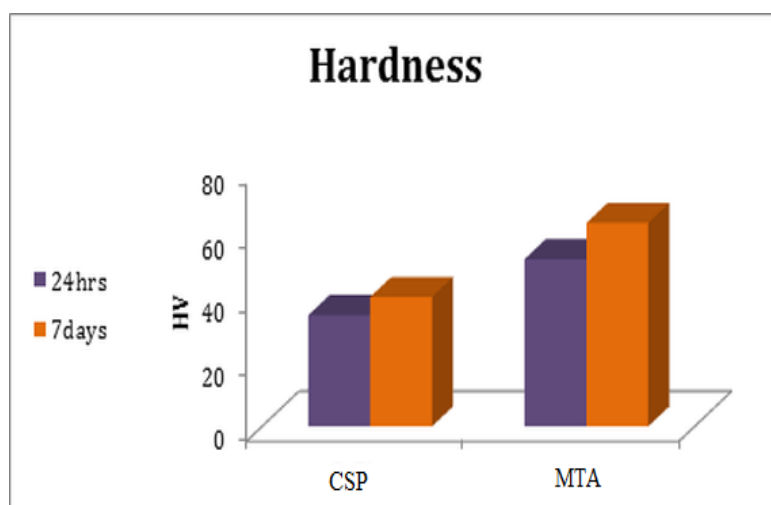

Fig. 5: Bar chart representing Microhardness results of each group.

Table (2): The mean, standard deviation (SD) values of Microhardness Vickers hardness units (VHN) of CSP and MTA.

Variables	CSP	MTA	P-value
	Mean ± SD	Mean ± SD	
24hrs	34.60 ± 8.14	52.02 ± 15.72	0.05ns
7days	40.24 ± 13.84	63.26 ± 15.93	0.04*
P-value	0.1ns	0.3ns	

CSP= Experimental Calcium silicate/phosphate cement.
MTA= Mineral Trioxide Aggregate
*; significant (p<0.05)   ns; non-significant (p>0.05)

DISCUSSION

In this study a new calcium silicate/phosphate cement was formulated for use as a root end filling to overcome some of the disadvantages of MTA, such as high cost, poor handling properties and delayed setting time[5,6]. C_3S phase was used as the main component (60% by weight) with 20% by weight TTCP to enhance the physical and mechanical properties of the synthesized biomaterial. C_3S phase hydrates immediately after mixing with distilled water resulting in a hardened structure. Curing of C_3S phase in an incubator at 37^oC enhances workability, wetting volume and hydration rate[13]. Adding bismuth oxide to the synthesized material provided adequate radiopacity and did not have any adverse effect on the hydration process. TTCP dissolute and hydrolyzes immediately after mixing with distilled water and HA layer forms on the surface of the anhydrous particles which will prevent further hydration. The less soluble HA layer will partly dissolute with the curing period and free water molecules will reach the anhydrous material resulting in liberation of more $calcium^{++}$ in the reaction medium[14]. C_3S cements have relatively longer setting times compared to calcium-phosphate cements. Calcium-chloride was used as the mixing liquid because of its low cost, availability, ability to accelerate both setting and hardening of the cement[21].

In this study we mixed the cement with distilled water first, then with different percentages of $CaCl_2$ (2, 4, 6 and 8 wt%). $CaCl_2$ had an accelerating effect on the hydration reaction of the cement pastes; it shortened the setting time. As seen in the setting time curve (Fig. 3), the use of 2 and 4% $CaCl_2$ mixing liquids showed quick decrease in setting time than with 6 and 8% solutions, this might be attributed to the common ion effect[15]. The 4% $CaCl_2$ solution that showed 4-min setting time was taken as the optimum mixing liquid for this investigation.

Dimensional linear change of the set mix was measured according to the ANSI/ADA specification no.57[18]. The experimental cement showed 0.16% expansion when mixed with distilled water and a 0.17% expansion when mixed with $CaCl_2$. These results are within the percentage recommended by the ANSI/ADA specification no.57, which stated that dimensional changes should not exceed 1%. Expansion may be due to mixing with $CaCl_2$ solution which enhances the hydration reaction rate of the synthesized composite cement. This accelerating effect promotes the hydration reaction to form additional amounts of hydrated compounds in larger volume, namely; calcium-silicate hydrate (C-S-H gel), hydroxyapatite (HA) and free lime [$Ca(OH)_2$]. In addition to the formation of more hydration products, the highly reactive $Ca(OH)_2$ released in the reaction medium reacts with CO_2 from the atmosphere resulting in the formation of $CaCO_3$ causing more expansion of the prepared pastes[13,15].

Digitized radiographic images are proved to be an easy, accurate and reproducible method to determine radiopacity of materials[22]. Several radiopacity studies have involved the use of an aluminium stepwedge. Shah et al, suggested that retrograde filling materials should be easily distinguished from adjacent bone and root dentin thus they should not be lower than 3-mm Al[23].

In the present study, CSP showed significantly higher radiopacity approximately 8-mmAl when compared to MTA 3.5-mmAl. This difference may be due to the presence of 20% bismuth-oxide in CSP composition. The lower values of radiopacity of MTA than other studies [24] may be due to the use of MTA Angelus rather than ProRoot MTA in this study, as MTA Angelus is known to have less radiopacity when compared to ProRoot MTA [25].

Ca^{++} released increased by time. The chemical reactions (eq.1&2), explained that the setting process of the two materials resulted in precipitation of hydration products; calcium-silicate hydrate (C-S-H), HA and $Ca(OH)_2$ immediately after mixing with distilled water. According to these chemical reactions, the two main phases of the prepared composite [C_3S and TTCP] are similar in that their hydration reactions liberate $Ca(OH)_2$ in the reaction medium causing an increase in calcium^{++} concentration with time[14].

Surface microhardness of a material is measured to give an indication about the degree of hydration the material has undergone during the setting reaction[26-29]. Microhardness was measured at 24hrs and 7 days with no significant difference between readings for both materials. Results showed that MTA had significantly higher hardness scores than CSP after 7 days this may be due to the presence of 20% TTCP Phase that has a relatively very weak mechanical properties compared to MTA. So, the presence of TTCP in the mix composition might have affected strength properties including surface hardness.

CONCLUSION

It was concluded that the experimental CSP had good physical and mechanical properties for use as a root end filling. Further leakage, adaptation and biocompatibility studies will be conducted in part II.

ACKNOWLEDGEMENT

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