Fabrication of fluorapatite cement as an anti-caries agent based on calcium phosphate cement

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ABSTRACT

Introduction: Many research proves that fluorapatite cement is one of the materials in dentistry that can restore and treat severe carious lesions. The current research was aimed to study the chemical reaction of calcium phosphate cement (CPC), calcite, and calcium fluoride as a base in fabricating the fluorapatite cement as an anti-caries agent. **Methods:** The type of research is a descriptive method. The preparation of DCPA was initially performed by measuring 0.2g of DCPA powder which was prepared for 15 samples. Fluorapatite was fabricated using dicalcium phosphate anhydrous (DCPA), calcite (CaCO3) as the precursor to form carbonate apatite crystal, and calcium fluoride (CaF2). CaF2 will be mixed into sodium dihydrogen phosphate (NaH2PO4) with different percentages, 1%, 5%, and 10%. The samples were then incubated for 24 hours at 37˚C under 100% moisture condition. X-Ray Diffraction (XRD) characterization will be conducted to determine the phase composition and crystallinity of fluorapatite. In addition, the samples were analyzed with the Fourier Transform Infrared Spectroscopy (FTIR) examination to determine the crystal functional group. **Results:** All of the samples showed a satisfactory setting reaction. The XRD characterization results showed the formation of the crystal apatite phase at 32.43˚ and 32.83˚. The FTIR results indicated the stretching vibration of the fluoride compound at 1400 cm-1. The intensity formation of the apatite crystal phase at particular degrees was not as high as the standard hydroxyapatite data due to the incomplete formation of apatite crystal within 24 hours—the stretching vibration presence in the FTIR graph at 1400 cm-1 proven to be fluorapatite cement. **Conclusions:** Fluorapatite cement as an anti-caries agent can be fabricated through the mixing process of DCPA, CaCO3, and CaF2 powders with NaH2PO4 solution.

Keywords: Fluorapatite cement; anti-caries agent; calcium phosphate cement;X-Ray Diffraction; Fourier Transform Infrared Spectroscopy.

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INTRODUCTION

Fluorapatite with the chemical formula of $Ca_5(PO_4)_3F$ is one of the apatite families with the most stable phase, least soluble in the physiological condition of human body fluid, with the hardest characteristics.[1,2,3] The precipitates with F are commonly and widely used in dentistry, mostly in restoration because of their anti-cariogenic characteristic, making dental enamel and dentin very resistant toward caries.[4] Equally important as to prevent caries activity, Al-Taie[5] was trying to develop dental composite materials containing fluorapatite as a filler as it is used in dental composite material to bring down the shrinkage that happens because of the curing, fluorapatite is corporate as secondary filler in the composite material to develop highly filled model resin composites. It was deduced that fluorapatite crystal has a unique morphology that augments the filler in the resin matrix compared to other commercial dental composites. Besides restoration, fluorapatite has been introduced as one of the components in ceramic scaffolds for bone tissue engineering in the oral surgery field. It was known that calcium phosphate-based ceramics are proven to maximize activation of cells and growth factors in the fabrication of bone tissue engineering scaffolds. Recent research added fluoride into calcium phosphate to produce fluorapatite-based ceramic, which is fluorapatite-glass ceramics. It is used to produce ceramic scaffolds for bone regeneration by augmenting the differentiation of human mesenchymal stem cells.[6,7]

Nowadays, many pulp capping materials have been introduced which all of them are composed of different kinds of cement and have many different advantages. Calcium hydroxide ($Ca(OH)_2$) has become a gold standard to be a pulp capping agent as it shows excellent biocompatibility towards pulp structure. Besides, deionization of $Ca(OH)_2$ has bactericidal effects.[8] However, $Ca(OH)_2$ exhibits the dissolution effects in tissue fluids, degradation on tooth flexure, and tunnel defects formation under dentinal bridges, making it fail to reach the objectives pulp capping to stimulate the reparative dentin formation and to prevent secondary caries.[9,10] A pulp capping method developed in 2012 using a single bond adhesive system shows various subclinical failures associated with pulp tissue.[11] Hence, we are trying to minimize the failure function of pulp capping to ensure the objectives of pulp capping can be achieved. Several factors must be focused on the cement type selection. The cement should have a good biocompatibility characteristic in which it should be able to adapt with the dental pulp and soft tissue. In addition, cement should have good physical and mechanical properties to ensure it could endure various forces and pH levels in the oral cavity.[12,13] A study has been conducted regarding the advantages of calcium phosphate cement (CPC). The result appears that CPC exhibits excellent biological properties as it could be a source of grafting application and inducing odontoblast cells in dentinal tissue.[14,15]

In this current research, for the first time, we were investigating the production of fluorapatite by preliminary test using the mass ratio of 60:40 of DCPA. Calcite will be mixed with a different concentration of Calcium Fluoride as the source of F^-. Therefore, the current research was aimed to study the chemical reaction of calcium phosphate cement (CPC), calcite, and calcium fluoride as a base in fabricating the fluorapatite cement as an anti-caries agent.

METHODS

The current research was conducted with a descriptive method to observe the X-Ray Diffraction (XRD) and Fourier Transform Infrared Spectroscopy's (FTIR) graph pattern. An experimental laboratory was conducted in the Integrated Laboratory of the Faculty of Dentistry Universitas Padjadjaran, Indonesia. XRD analysis was performed at the Faculty of Mining and Petroleum Engineering Bandung Institute of Technology, Indonesia, while the FTIR analysis was performed at the Faculty of Pharmacy Universitas Padjadjaran, Indonesia, from March until May 2019.

The materials used were dicalcium phosphate anhydrous (DCPA) powder, calcite powder, distilled water, and 1, 5, and 10% of calcium fluoride (CaF_2) powder diluted in sodium dihydrogen phosphate (NaH_2PO_4) solution. This mixture was expected to produce the fluorapatite paste. The samples were then analyzed through the X-Ray Diffraction (XRD) and Fourier Transform

Infrared Spectroscopy (FTIR) to determine the phase composition and IR spectrum. The instruments used in this research were digital scale, measuring cylinder, beaker glass, magnetic stirrer, hot plate, lab glass, spatula, Teflon mold (6mm x 3mm), C-clamp, and incubator (Memmert Incubator IN 30).

The preparation of DCPA was initially performed by measuring 0.2g of DCPA powder which was prepared for 15 samples. Afterwards, the preparation of 1, 5, and 10% of calcium fluoride in 0.2M of sodium dihydrogen phosphate solution was conducted by measuring 0.5g of calcium fluoride powder in a measuring cylinder, then diluted in 50ml of sodium dihydrogen phosphate solution in a beaker glass. This step was repeated with different amounts of calcium fluoride powder (2.5g and 5.0g) to create different solution concentrations.

The fluorapatite paste was created by initially mixing 0.2g of DCPA powder. The calcite powder was diluted with 0.1ml of 1% calcium fluoride in sodium dihydrogen phosphate solution. The paste created was then packed into a 6mm x 3mm Teflon mold. Both ends of the mold were then covered with glass plates held by a C-clamp, then incubated for 24 hours with 100% relative humidity box at 37˚C. Another 4 samples were created with the same steps as prior, and all sample pastes were diluted in 5 and 10% calcium fluoride in sodium dihydrogen phosphate solution. After 24 hours of incubation, the samples were taken out and dipped into an ethanol solution for 3 minutes, then dried in the incubator for 3 hours. All samples were subjected to an XRD analysis to determine the crystallization phase and FTIR analysis to determine each of the functional groups' vibration in the sample.

RESULTS

The experiment was conducted to fabricate the samples within 24 hours, as suggested in Table 1 and Figure 1.

Table 1. The positive result of the samples within 24 hours

Sample	Sample A					Sample B					Sample C				
Setting reaction (+/-)	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

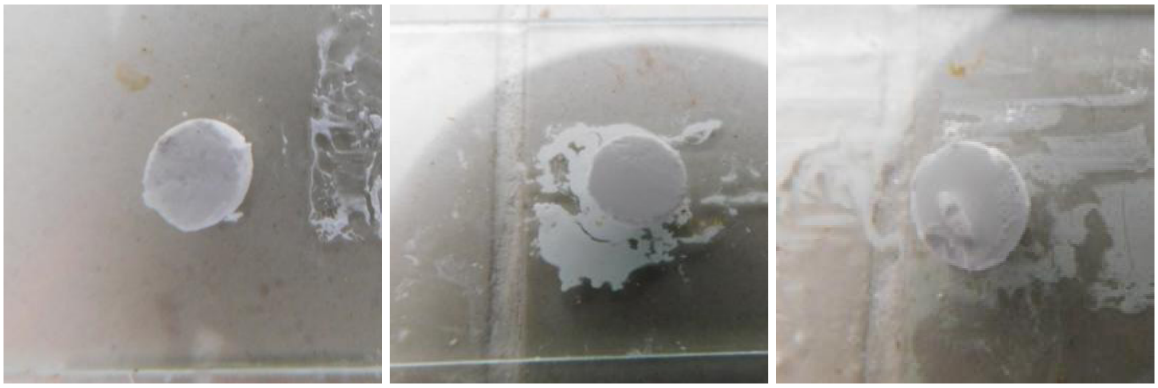

Figure 1.A. Sample A manage to set within 24 hours; B. Sample B manage to set within 24 hours; C. Sample C manage to set within 24 hours

The samples were then being taken for the X-Ray Diffraction process to examine the XRD graph qualitative data. This experimental XRD data was made and dictated with the reference XRD graph.[16] In this case, a hydroxyapatite (HAp) XRD graph was used to dictate what phases existed in the sample. The data of the HAp XRD graph was based on International Centre for Diffraction Data[17], and presented as the comparison of the XRD graph between the ICDD hydroxyapatite and fluorapatite sample (Figure 2).

The XRD pattern shown in Figure 2 showed the differences between standard hydroxyapatite data and fluorapatite XRD pattern obtain from the sample as apatite crystal was observed in standard hydroxyapatite data at phase 32.26˚ and

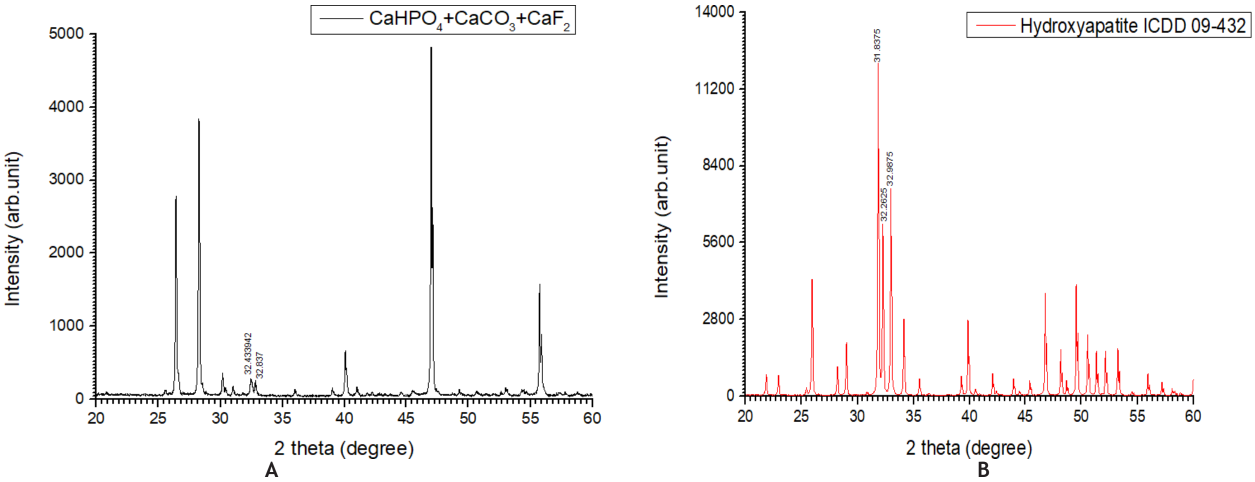

Figure 2A and B. Comparison of XRD graph between ICDD hydroxyapatite and fluorapatite sample

32.98˚, which were almost at the same phase with fluorapatite sample at 32.43˚ and 32.83˚. The intensity of the diffraction in the phase indicated that the apatite crystal in the fluorapatite sample was lower than the standard hydroxyapatite data. The FTIR spectrum result, which observed the absorption peaks of the sample characteristic, was presented in Figure 3.

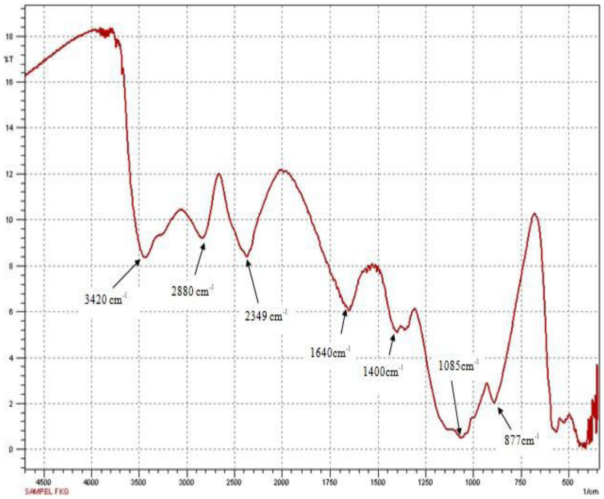

Figure 3. The spectrum of F^- at 1400 cm^{-1}

Table 2. Absorption of common functional groups

Functional groups	Absorption location (cm^{-1})	Absorption intensity
Fluoro compound	1400	Strong
Carbonate compound	877, 1050, 1650	Strong
Phosphate Compound	1085, 1640	Sharp shoulder
Carbon dioxide	2349	Strong
Water	3200-3550	Strong

The FTIR spectrum presented in Figure 3 showed the characteristic absorption peaks of sample fluorapatite based on Table 2. A broad band at 1400 cm-1 indicates the stretching vibration of F^- while CO_{3-} at 877cm^{-1}, 1050 cm-1 and 1650 cm-1. The characteristic of the CO2 band appeared in 2349cm^{-1} while PO_{3-} at 1085 cm-1 and 1640 cm-1. A strong and broad carboxylic acids functional group is detected at 2880cm^{-1} besides an alcohol functional group at 3420cm^{-1}.[18,19]

DISCUSSION

Alot of pulp capping agents are being invented to fulfill the functions with several advantages in each of the materials, including fluorapatite cement. Fluorapatite cement is a new alternative for pulp capping agents that is claimed to has excellent biocompatibility.[20] Furthermore, CPC associated with carbonate apatite can induce osteoblast cells in dental, craniofacial, and other orthopedic implements.[21] In the future, the formation of fluorapatite produced by this research could be used in the form of cement in order to repair the enamel defects as well. In 2010, research had been done involving a fluoride form of crystalline apatite cement paste that could be applied directly to the cavities.[22] The mixture of the paste is done by mixing the fluorapatite powder with cement liquid which is water with a different ratio of powder to liquid (P/L), and the hardened cement is defined as the fluorapatite. On the other hand, research conducted in Beijing Peking University fabricated fluorapatite cement mixed with diluted phosphoric acid (H_3PO_4). It claimed to have the mixture which can be directly applied into the carious cavity to repair the defects at the teeth enamel.[23]

Fluorapatite is claimed to be one of the apatite family members, which has the most stable phase, is least soluble in the physiological condition of human's body fluid, and has the hardest characteristics. In this research, we try to mix DCPA, $CaCO_{3,}$ and different percentages of CaF_2 in NaH_2PO_4 solution to produce fluorapatite paste that can be applied for pulp capping material. According to XRD and FTIR results, it is shown that the hardened paste is fluorapatite cement since the presence of apatite crystal in the XRD result and fluor compound in the FTIR result are detected. The presence of fluor compound in the hardened paste is the main component that makes the cement the anti-caries agent. The combination of it with the apatite crystal producing fluorapatite results in the cement being resistant to the oral environment's critical pH[1,2,3] This advantage of fluorapatite could endure with critical pH of the oral environment so that its usage might be long-lasted.

The apatite phase of the crystal attributed its presence in the XRD graph in figure IV-5 (a) for the fluorapatite sample. The differences of the X-Ray intensity in fluorapatite sample and standard hydroxyapatite data is observed that fluorapatite sample has lower intensity compared to standard hydroxyapatite data in the same degree of diffraction. This might occur because of the incomplete formation of the apatite crystal within 24 hours. Cahyanto, in his research, proven that the use of NaH_2PO_4 as a liquid for the mixture of carbonate apatite powder consists of DCPA and $CaCO_3$ needs 96 hours of incubation for a complete formation of apatite crystal.[24]

FTIR is used in this research to indicate chemical bonds by producing an infrared absorption spectrum. The vibration produced will identify the compound present in the sample. In this research, after the samples undergo FTIR, several compounds could be detected, as clarified in table IV-2. The stretch vibration of F^- at 1400cm^{-1} to the anti-caries agent's characteristic of the sample while the stretch vibration of CO_3^- at 877 cm^{-1}, 1050 cm$^{-1,}$ and 1650 cm^{-1}. Phosphate compounds can be detected at 1085 cm$^{-1,}$ and carbon dioxide compounds found at 2349 cm^{-1}. The stretch vibration at 3200 cm^{-1} to 3550cm^{-1} attributes to the absorbed water.

$$6 \quad CaHPO_4\ (s) + CaF_2\ (l) + \ 3 \quad CaCO_3\ (s) + 9O_2\ NaH_2PO_4\ 2\ Ca_{10}(PO_4)_6F_2\ (s) + 3\ CO_2 + 3\ H_2O$$

When the mixture process occurs, the ionization process will happen as well. F^- and CO_3^- will compete with each other to attract $Ca3(PO_4)_{2.}$ It is concluded from the above equation that F^- from CaF_2 will attach to the hydroxyapatite crystal in $Ca_5(PO_4)_3OH$ instead of CO_3^- because of the high reactivity of F^- compared to CO_3^-. The reactivity of these ions will be explained further by the periodic table of elements.[25] When further across to the right of the periodic table, the reactivity of the non-metal elements will increase as the non-metal elements tend to gain electrons. As F^- is located further away to the right compared to CO_3^-, F^- is expected to be attracted towards apatite ions instead of CO_3^-.[26,27]

The presence of CO_3^- in the mixture could contribute as the buffer for fluorapatite paste as the liquid use has acidic properties. The critical pH for enamel is 5.5 while 6.2 for dentin, in which tooth layers are sensitive in acidic conditions. As the pH value in the oral cavity drops below the critical pH of enamel and dentin, this might cause the demineralization of the tooth and necrosis towards the pulp. Hence, the ionization of calcite would naturalize the acidic pH of the paste.[28]

Fluorapatite cement might be possible to be used as an alternative therapy for pulp capping agent besides Mineral Trioxide Aggregate (MTA) and calcium hydroxide [$Ca(OH)_2$] because of its ability to remain in the oral cavity to prevent secondary caries on research stating that $Ca(OH)_2$ it is very soluble if contaminated with any liquids including water. Furthermore, $Ca(OH)_2$ has a high pH reaching 12.5, which might cause irritants and necrosis towards pulp tissue.

Samples that undergo setting reactions need to be incubated for a more extended period, at least 72 hours, to ensure the complete formation of apatite crystal. It is also suggested to continue the research regarding the setting time of fluorapatite cement to resolve the disadvantage of this research where the structure of the samples that are expected to undergo setting reaction is not satisfactorily following the shape of the mold used.

CONCLUSIONS

Fluorapatite cement could be produced by mixing DCPA, $CaCO_3$, and CaF_2 powders with NaH_2PO_4 liquid.

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REFERENCES

1. Jokanovic V, Colovic B, Sandic-Zivkovic M, Popovic Bajic M, Zivkovic Z. Structural characteristics and mechanisms of fluorapatite mechanochemical synthesis. Serbian Dent J. 2016; 63(2): 74–9. DOI: 10.1515/sdj-2016-0008
2. Taheri MM, Shirdar MR, Keyvanfar A, Shafaghat A. Evaluating hydrothermal synthesis of fluorapatite nanorods: ph and temperature. J Exp Nanosci. 2017; 12(1): 83–93. DOI: 10.1080/17458080.2016.1263400
3. Wei S, Wei J, Liu X, Wang C, Zhang Y. Method for Producing Fluorapatite and Its Application. Patent Application Publication. China: United States; PCTPCN201 0/001336, 2012. p. 1-6.
4. Junpoom P, Kukiattrakoon B, Hengtrakool C. Flexural strength of fluorapatite-leucite and fluorapatite porcelains exposed to erosive agents in cyclic immersion. J Appl Oral Sci. 2011; 19(2): 95–9. DOI: 10.1590/S1678-77572011000200003
5. Al-Taie AMBJ. Development and Characterization of Novel Fluorapatite Containing Dental Composite Materials. The University of Leeds School of Dentistry; 2017.
6. Isabelle D, Ourania-Menti G, Fredericks DC, Akkouch A, Acevedo MR. Holloway JA. Strontium-releasing fluorapatite glass-ceramics scaffolds: Structural characterization and in vivo performance. Acta Biomater. 2018; 75:463–71. DOI: 10.1016/j.actbio.2018.05.047
7. AsiriAM, Inamuddin, MohammadA. Fluorapatite-based Glass Ceramic. In: Applications of Nanocomposite Materials in Dentistry. Massachusetts: Elsevier Inc.; 2019. p. 13.
8. Rezapour M. Comparison of the Effects of Different Pulp Capping Materials on Viability, Morphology, and Ageing of Dental Pulp Cells. England; 2015.
9. Qureshi A, Soujanya E, Nandakumar, Pratap Kumar, Sambashivarao. Recent Advances of Pulp Capping Materials: An Overview. J Clin Diagnostic Res. 2014;8(1):316-21. DOI: 10.7860/JCDR/2014/7719.3980.
10. Lipski M, Nowicka A, Kot K, Postek-Stefanska L, Wysoczanska- Jankowicz I, Borkowski L, et al. Factors affecting the outcomes of direct pulp capping using Biodentine. J Clin Oral Investig. 2018;22(5):2021–9. DOI: 10.1007/s00784-017-2296-7.
11. Silva GAB, Gava E, Lanza LD, Estrela C, Alves JB. Subclinical failures of direct pulp capping of human teeth by using a dentin bonding system. J Endod. 2013;39(2):182–9. DOI: 10.1016/j.joen.2012.09.022.
12. Yu H, Zheng M, Chen R, Cheng H. Proper selection of contemporary dental cements. J Oral Heal Dent Manag. 2014;13(1):54-9.
13. Goncalves SEP, Bresciani E. Reconstructions using Alloys and Ceramics. In: Material -Tissue Interfacial Phenomena. Cambridge: Woodhead Publishing; 2017. p. 23-66.
14. Shimatani A, Toyoda H, Orita K, Ibara Y, Yokogawa Y, Nakamura H. A bone replacement-type calcium phosphate cement that becomes more porous in vivo by incorporating a degradable polymer. Biomaterials Synthesis and Characterization 2021;32(77):335.
15. Kim J-W, Kim S-W, Kim G-C. Response of odontoblast to the bio- calcium phosphate cement. J Korean Assoc Maxillofac Plast Reconstr Surg. 2011;33(4):301-7. DOI: 10.1186/s12877-019-1198-8.
16. Inagaki M. X-Ray Powder Diffraction. In: Materials Science and Engineering of Carbon. 2016. p. 7-25. DOI: 10.1016/B978-0-12-805256-3.00002-7.
17. Hydroxyapatite XRD database [Internet]. International Centre For Diffraction Data. 2018 [cited 2019 Jan 16]. Available from: http://www.icdd.com

18. IR Spectrum Table & Chart [Internet]. Sigma-Aldrich Inc. 2019 [cited 2019 Apr 2]. Available from: http://www.sigmaaldrich.com
19. Singh K, Li G, Lee J, Zuilhof H, Beata L. Mehdi, Rafael L, et al. Divalent Ion selectivity in capacitive deionization with vanadium hexacyanoferrate: experiments and quantum-chemical computations. Adv Funct Mater 2021;2105203:1-13. DOI: 10.1002/adfm.202105203.
20. Dorozhkin SV. Self-Setting Calcium Orthophosphate Formulations: Cements, Concretes, Pastes and Putties. Inter J Mater Chemis 2011;1(1):1–48. DOI: 10.5923/j.ijmc.20110101.01.
21. Wang P, Zhao L, Chen W. Stem Cells and Calcium Phosphate Cement Scaffolds for Bone Regeneration. J Dent Res. 2014;93(7):618-25. DOI: 10.1177/0022034514534689.
22. Wei J, Wang J, Shan W, Liu X, Ma J, Liu C, et al. Development of Fluorapatite Cement for Dental Enamel Defect Repair. J Mater Sci. 2011;22(6):1607-14. DOI: 10.1177/0022034514534689.
23. Acton QA. Peking University, Beijing: Preparation of Fluoride Substituted Apatite Cement as the Building Blocks for Tooth Enamel Restoration. In: Advances in Hydrofluoric Acid Research and Application. Georgia: Scholarly Edition; 2012. p. 164.
24. Cahyanto A, Maruta M, Tsuru K, Matsuya S, Ishikawa K. Basic Properties of Carbonate Apatite Cement Consisting of Vaterite and Dicalcium Phosphate Anhydrous. Key Eng Mater. 2013;529–530:192–6. DOI: 10.4028/www.scientific.net/KEM.529-530.192.
25. 71Periodic Table of the Element [Internet]. Sigma-Aldrich Inc. 2019 [cited 2019 Mar 20]. Available from: http://www.sigmaaldrich.com.
26. Francis E. Lesson 6: Reactivity Trends (Lab Work) [Internet]. Clackamas Community College. 2011 [cited 2019 Mar 20]. Available from: http://www.dl.clackamas.edu
27. Yang WH, Xi XF, Li JF, Cai KY. Comparison of crystal structure between carbonated hydroxyapatite and natural bone apatite with theoretical calculation. Asian J Chemis 2013; 25(7): 3673-8. DOI: 10.14233/ajchem.2013.13709.
28. Koenigs PM, Faller R V. Fluoride's Mechanism of Action. dentalcare.com. 2019.