International Journal of Chemical, Materials and Biomolecular Sciences
ISSN: 2415-6620
Vol:9, No:12, 2015

The Effects and Interactions of Synthesis Parameters on Properties of Mg Substituted Hydroxyapatite

S. Sharma, U. Batra, S. Kapoor, A. Dua

***Abstract*—**In this study, the effects and interactions of reaction time and capping agent assistance during sol-gel synthesis of magnesium substituted hydroxyapatite nanopowder (MgHA) on hydroxyapatite (HA) to β-tricalcium phosphate (β-TCP) ratio, Ca/P ratio and mean crystallite size was examined experimentally as well as through statistical analysis. MgHA nanopowders were synthesized by sol-gel technique at room temperature using aqueous solution of calcium nitrate tetrahydrate, magnesium nitrate hexahydrate and potassium dihydrogen phosphate as starting materials. The reaction time for sol-gel synthesis was varied between 15 to 60 minutes. Two process routes were followed with and without addition of triethanolamine (TEA) in the solutions. The elemental compositions of as-synthesized powders were determined using X-ray fluorescence (XRF) spectroscopy. The functional groups present in the as-synthesized MgHA nanopowders were established through Fourier Transform Infrared Spectroscopy (FTIR). The amounts of phases present, Ca/P ratio and mean crystallite sizes of MgHA nanopowders were determined using X-ray diffraction (XRD). The HA content in biphasic mixture of HA and β-TCP and Ca/P ratio in as-synthesized MgHA nanopowders increased effectively with reaction time of sols ($p<0.0001$, two way ANOVA), however, these were independent of TEA addition ($p>0.15$, two way ANOVA). The MgHA nanopowders synthesized with TEA assistance exhibited 14 nm lower crystallite size ($p<0.018$, 2 sample t-test) compared to the powder synthesized without TEA assistance.

***Keywords*—**Capping agent, hydroxyapatite, regression analysis, sol-gel, 2- sample t-test, two-way ANOVA.

I. Introduction

WITHIN the last four decades a revolution has occurred in the innovative use of specially designed ceramics for the repair and reconstruction of a diseased or damaged part of the bone [1]. Synthetic HA, [Ca_{10} $(PO_4)_6(OH)_2$] has widely been used for repairing hard tissues due to its chemical and structural similarity to the mineral phase of bone and tooth [2]. This inorganic phosphate has been studied extensively for medical applications in the form of powders, composites, and coatings [3]. Biological apatite contains trace ions like Na^+, Mg^{2+} and K^+, which are essential in biological processes. Magnesium plays a key role in bone metabolism as it affects osteoblast and osteoclast activity and thereby bones growth [4]. Mg^{2+} ion stabilizes the crystal lattice of HA [5]. The properties and applications of synthetically prepared HA are influenced by the size and morphological characteristics of their particles [6]. The crystallite size of HA for adult enamel is 130 nm, for dentine is 20 nm and for bone is 25 nm [7]. Published work show that the synthesis procedure, control of experimental parameters and reagents used influence the resulting crystallite size. In addition, the process parameters also cause change in the constituent phases and Ca/P ratio of the final product. The Ca/P ratio of HA and β-TCP is 1.67 and 1.50, respectively and for a biphasic mixture consisting of HA and β-TCP, it is between 1.50-1.67. In order to have uniform chemical composition and particle size, sol-gel synthesis route was selected for present study.

The HA powder with different phase constituents and sizes find different implications in biological applications. In view of this, the present investigation deals with the study on the influence of reaction time of sols and capping agent (TEA) assistance on the HA/β-TCP ratio, Ca/P ratio and crystallite size of resulting MgHA powders through experimental results and their statistical analysis using 2 sample t-test and two way ANOVA.

S. Sharma and S. Kapoor are with the University Institute of Chemical Engineering and Technology, Panjab University, Chandigarh-160014, India (e-mail: soniaphd@yahoo.in, seemakapoor@pu.ac.in).

U. Batra and A. Dua are with the Dept. of Materials and Metallurgical Engineering, PEC University of Technology, Chandigarh-160012, India (phone: 91-0172-2753090, e-mail: umabatra2@yahoo.com, dua_ankit09@yahoo.co.in).

II. Materials and Methodology

A. Synthesis of HA with and without TEA

To synthesize MgHA nanopowders ($Mg_zCa_{10-z}(PO4)_6OH_2$, $z = 0.2$), calcium nitrate tetrahydrate (CNT, $Ca(NO_3)_2.4H_2O$, Merck, AR grade) and potassium dihydrogen phosphate (KDP, KH_2PO_4, Merck, AR grade) were used as precursors of calcium and phosphorous, respectively. Magnesium nitrate hexahydrate (MNT, $Mg(NO_3)_2.6H_2O$, Merck AR grade) was used as magnesium precursor. 1.0 M solution A was prepared by mixing 0.98 M of aqueous solution of CNT and 0.02 M aqueous solution of MNT. The Solution A was then added drop-wise to 0.6 M KDP solution under vigorous stirring at 2100 rpm for 1 hour at 10°C. (Ca+Mg)/P molar ratio was maintained at 1.67. The pH was continuously monitored and adjusted to 10±0.1 by adding 25% NH_4OH solution to improve gelation and polymerization of HA structure. MgHA nanopowder with TEA assistance was synthesized by adding 0.6 M TEA (Merck, India) into KDP and then following the procedure as explained above. The molar ratio of CNT + MNT: TEA was maintained at 1:0.6.

During the mixing and stirring step, a part of sol mixture was withdrawn at regular interval of 5 minutes starting from 15 minutes till 60 minutes and then aged for 24 hours at 25±2°C. Gelatinous precipitates thus formed were centrifuged and washed thoroughly by double distilled lukewarm water. The precipitates were dried in an oven at 70°C for 48 hours. The dried mass from above step was crushed and ground with

the help of a mortar and pestle to obtain fine powders. As-synthesized MgHA nanopowders were calcined for 2 hours in a silicon carbide programmable muffle furnace at a heating rate of 5°C/min. at 900°C and finally cooled in furnace by switching it off.

B. Powder Characterization

The elemental analysis (by weight) of MgHA powders was carried out by using wavelength dispersive X-ray Fluorescence Spectroscopy (WD-XRF, Model: S8 TIGER, Make Bruker, Germany). Approximately 8.0 grams of powder was pressed at a load of 15 tons using hydraulic press to form pellets of 34 mm diameter and 1.5 mm thickness. The pellets were analyzed for 17 minutes in WD-XRF. XRF spectra were obtained for determination of elemental concentration in powders. Infrared spectra (FTIR, Perkin Elmer) were recorded in the region 450–4000 cm^{-1} using KBr pellets (1% wt/wt), with spectral resolution of 2 cm^{-1} for the nanopowders by taking four scans for each sample. The spectra were analyzed to identify the various functional groups such as hydroxyl, phosphates, nitrates and carbonates present in nanopowders. XRD, Philips X'Pert 1710 was used for XRD analysis. The test was performed for MgHA powder using CuKα radiation, λ = 1.54 Å, 2θ- 20˚ to 90˚, step size 0.017°, time per step 20.03 s and scan speed 2.12°/min. XRD analysis was carried out to determine the phases, mean crystallite size and lattice parameters. Crystallite size of nanopowders was calculated using Scherrer's equation [8], [9].

$$X_s = \frac{0.9\lambda}{\beta \cos \theta} \quad (1)$$

where X_S is the crystallite size in nm, λ is the wave length of X-ray beam, β is the broadening of diffraction line at half of its maximum intensity in radians and 2θ is the Bragg's diffraction angle (^{o}). The silicon standard was used to measure the instrument broadening in order to correct the value of β. To calculate mean crystallite size of nanopowders, three peaks of XRD spectra which were well separated and had high intensities were chosen.

As reported by [10]-[12], thermal decomposition of calcium deficient hydroxyapatite, CDHA ($Ca_{10-x}(HPO_4)_x(PO_4)_{6-x}(OH)_{2-x}$) results in the formation of a biphasic mixture consisting of HA and β-TCP phases at temperatures above 800°C according to:

$$Ca_{10-x}(HPO_4)_x(PO_4)_{6-x}(OH)_{2-x} \xrightarrow{(1-x)} Ca_{10}(PO_4)_6(OH)_2 + 3xCa_3(PO_4)_2 + xH_2O \quad (2)$$

where, x is the calcium deficiency and $\frac{Ca}{P} = \frac{10-x}{6}$. The ratio of mole fraction of β-TCP to mole fraction of HA in the calcined samples is given by (3):

$$\frac{X_{\beta-TCP}}{X_{HA}} = \frac{3x}{(1-x)} \quad (3)$$

$$\frac{X_{\beta-TCP}}{1-X_{\beta-TCP}} = \frac{3x}{(1-x)} \quad (4)$$

The weight fractions of hydroxyapatite phase (W_{HA}) and β-TCP phase ($W_{\beta\text{-TCP}}$) estimated from XRD patterns using external standard method were converted into mole fractions and then used for calculating x and Ca/P values.

The two way ANOVA test was applied to analyze the effect of reaction time on HA/ β-TCP and Ca/P ratios of nanopowders. Two sample t test using Minitab software was performed to predict the effect of TEA on mean crystallite size of resulting MgHA nanopowder.

III. Results and Discussion

A. XRF Spectroscopic Analysis

The energy dispersive spectra of MgHA nanopowders synthesized by reaction of sol for 15 minutes with and without TEA assistance were used to determine the amount of Ca, Mg and P in nanopowders. The MgHA nanopowder synthesized with and without TEA assistance consisted of 0.13 wt.% and 0.21 wt.% of Mg, respectively. This confirmed substitution of Mg in hydroxyapatite at an early stage of synthesis.

Figs. 1 (a) and (b) show the FTIR spectrum of MgHA powders synthesized by reaction of sol ranging from 15 to 60 min. with TEA assistance and without TEA, respectively. All characteristic peaks of hydroxyapatite in the spectra i.e. phosphate vibrations of apatite:v_1 PO_4^{3-} (962 cm^{-1}), v_2 PO_4^{3-} (473 cm^{-1}), v_3 PO_4^{3-} (broad band 1031-1093 cm^{-1}) and v_4 PO_4^{3-} (569 cm^{-1} and 602 cm^{-1}); and hydroxyl group of hydrogen bonded to OH^-at 3569 cm^{-1} and 631 cm^{-1}were present in MgHA powders. The weak absorption peak at 868.7cm^{-1} in MgHA powder was ascribed to P–O–H vibration in HPO_4^{2-} group, typical of CDHA [13]. The stretching vibration of CO_3^{2-} at around 1400.3 cm^{-1} was detected. The incorporation of carbonate ions in the apatite structure might have taken place during the synthesis of HA, where the atmospheric CO_2 reacted with OH^- in the HA, forming carbonate ions [14]. Bone apatite contains 2-6 wt.% carbonate which commonly substitutes for PO_4^{3-} group in the HA structure [15]-[18]. As reported earlier CO_3^{2-} presence can improve the bioactivity of HA [17].

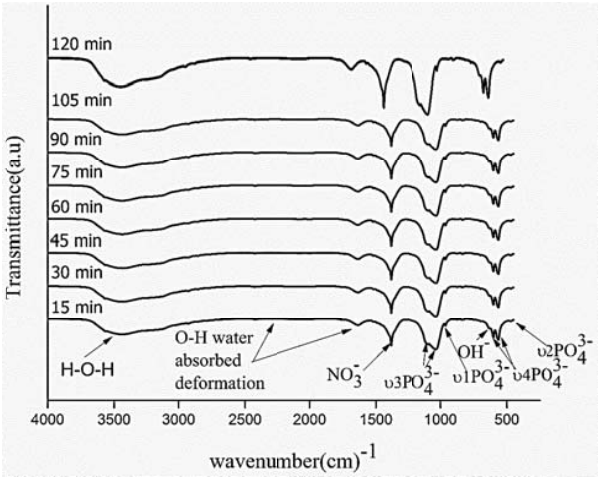
Broad envelop around 3400 $cm^{-1}$and 1648 cm^{-1} was ascribed to the O-H and H-O-H vibrations of the absorbed water molecules on the HA crystal structure, respectively. As the reaction time of sol was increased from 15 to 60 min., strength of $PO_4^{3-}$and OH^- bands in resulting powders increased while that of HPO_4^- band decreased.

B. XRD Analysis

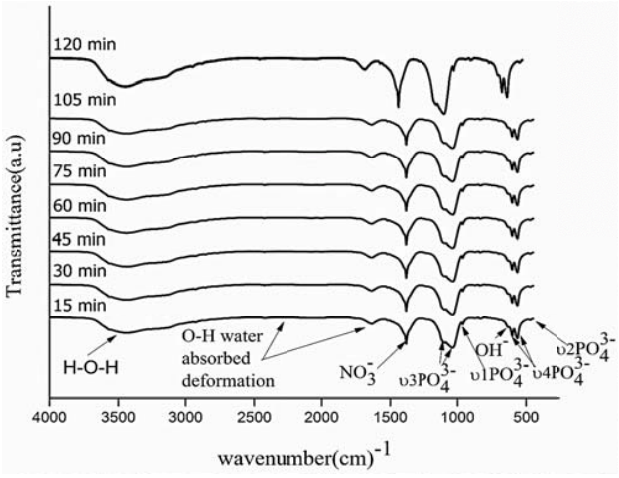
Figs. 2 (a) and (b) show the XRD patterns of MgHA powders synthesized by reaction of sol ranging from 15 to 60 min. with TEA assistance and without TEA, respectively. The lattice parameters of TEA assisted MgHA powder changed from 9.42 to 9.46Å (for a) and 6.82 to 6.86 Å (for c) and 1816 to 1833 [Å] ^{3} (for volume of HA unit cell) and the mean crystallite size changed from 8.75 to 29.43 nm as the reaction time was increased from 15 to 60 minutes.

For MgHA powder synthesized without TEA, as the reaction time was increased from 15 to 60 min. the lattice parameters changed from 9.35 to 9.46 Å (for a) and 6.82 to 6.88 Å (for c) and 1800 to 1854 $[Å]^3$ (for volume of HA unit cell) and the mean crystallite size changed from 9.53 to 40.99 nm.

According to Singh and Khanduja, the consistency of result can be predicted with 95% confidence using a hypothetical test known as '2 sample t-Test' [19]. Test was executed by using Minitab-16 software and its output is reported in Fig. 3. Necessary hypothecation was formulated by assuming *Null Hypothesis* as 'no effect of TEA (H_0: $\mu_1=\mu_2$)' whereas *Alternate Hypothesis* as 'significant effect of TEA (H_a: $\mu_1\neq\mu_2$)'. Here μ_1 and μ_2 were mean particle sizes of MgHA nanopowders synthesized with and without TEA assistance. For this analysis, the significance level is 0.05.

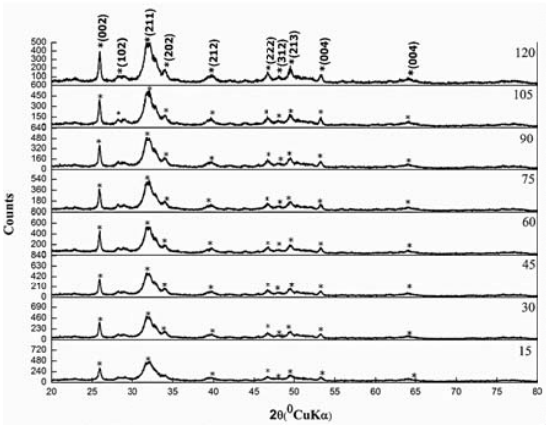


(a)

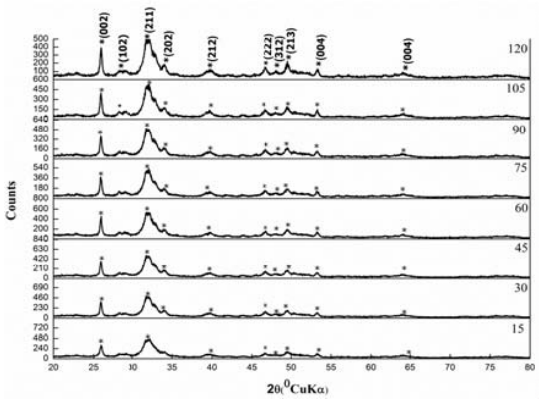


(b)

Fig. 1 FT-IR pattern of MgHA synthesized (a) with TEA assistance and (b) without TEA



(a)



(b)

Fig. 2 XRD pattern of MgHA synthesized from 15to 60 min. (a) with TEA assistance and (b) without TEA

Two-Sample t-Test :
Average Particle Size With Capping Agent & Without Capping Agent

Two-sample T for With Capping agent vs Without Capping Agent

	N	Mean	StDev	SE Mean
With Capping agent	10	22.68	5.93	1.9
Without Capping Agent	10	33.6	11.3	3.6

Difference = mu (With Capping agent) - mu (Without Capping Agent)

Estimate for difference: -10.90
95% CI for difference: (-19.64, -2.15)
T-Test of difference = 0 (vs not =): T-Value = -2.69
P-Value = 0.018 DF = 13

Fig. 3 Two Sample t-Test

Since the p value (0.018) was less than significance level, it was predicted with 95% confidence level that mean crystallite size for MgHA nanopowder synthesised with TEA assistance was different from that without TEA. The standard deviation value suggested the degree of variability in mean crystallite size of MgHA nanopowder during synthesis. Higher standard deviation value (11.3) for MgHA nanopowder synthesised without TEA suggested higher unpredictability (almost double) than lower standard deviation (5.93) for powder

synthesised with TEA assistance. The individual value plot was obtained using Minitab software to clearly illustrate the characteristics of data distribution graphically as shown in Fig. 4. Accordingly, the mean crystallite size of MgHA powder synthesized with TEA assistance and that without TEA were 22.68 nm and 33.58 nm, respectively.

The diagnostic report from the above plot given in Fig. 5 revealed that mean crystallite size of MgHA powder synthesized with TEA assistance was 14.15 nm higher than powder synthesized without TEA.

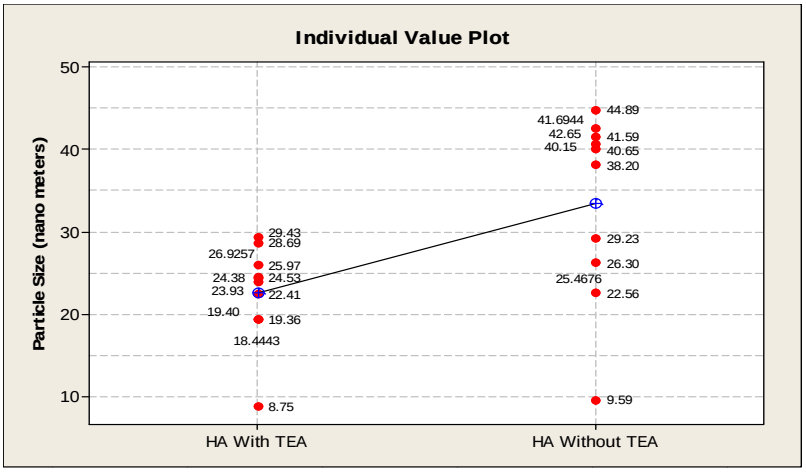


Fig. 4 Individual Value Plot for Nano Size Variation

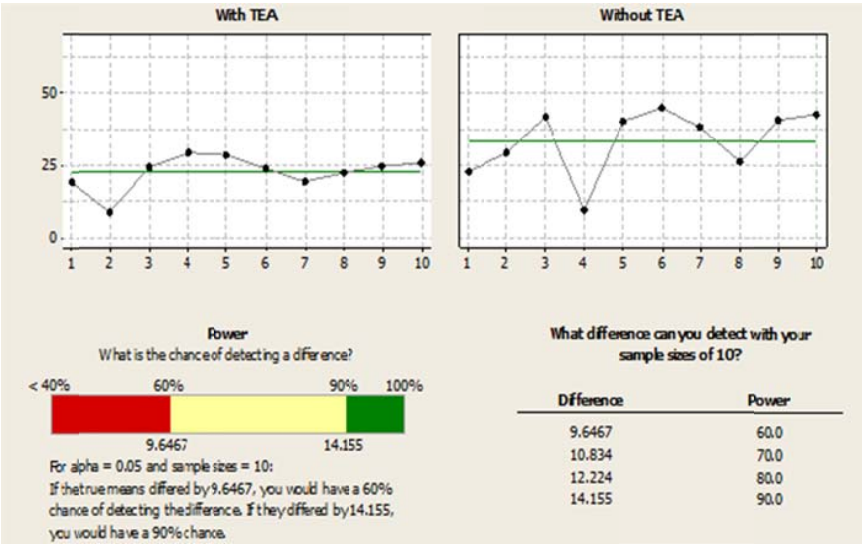


Fig. 5 Diagnostic Report for Nano Size Variation

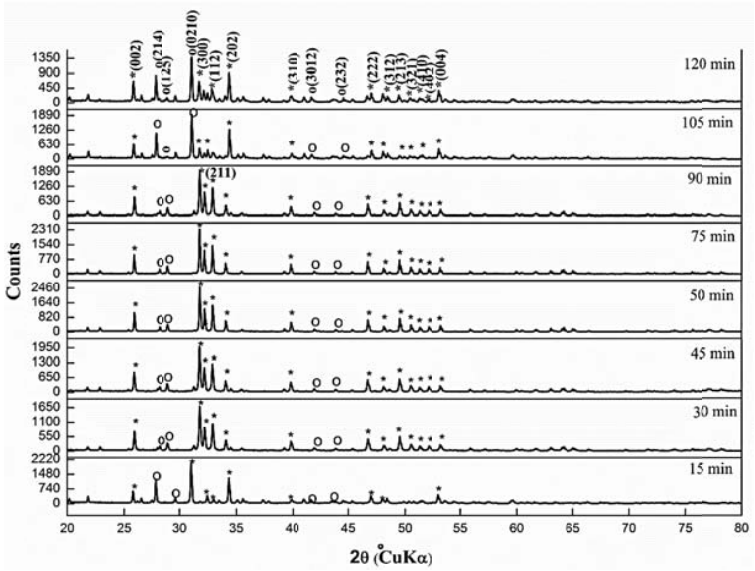
C. HA to β-TCP Ratio

Figs. 6 (a) and (b) show XRD patterns of calcined MgHA powders synthesized for reaction time from 15 to 60 min. with TEA assistance and without TEA, respectively.

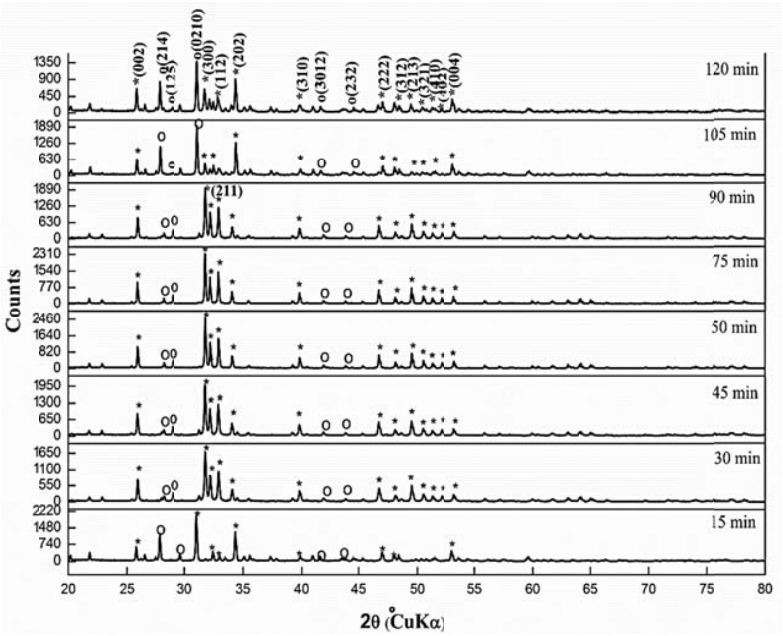
All calcined MgHA powders consisted of biphasic mixture of HA and β-TCP phases. The HA/β-TCP ratio decreased with an increase in reaction time from 15 to 60 min. The influence of reaction time on HA proportion present in biphasic mixture of HA and β-TCP in calcined MgHA nanopowders was obvious but the degree varied nanopowders synthesized with and without TEA assistance.

A 2-way ANOVA was used to test the HA proportion in biphasic mixture of HA and β-TCP for reaction time from 15 to 60 minutes in MgHA nanopowders synthesized with and without TEA assistance. Fig. 7 provides the summary of the results from 2-way ANOVA. The Anova showed that TEA had insignificant effect on HA proportion as p value obtained was greater than the significance level 0.05 ($p = 0.095$) whereas the reaction time had significant effect as $F = 60.09$ and $p < 0.05$. The HA proportions in biphasic mixture of HA and β-TCP in calcined MgHA nanopowder increased significantly when reaction time was increased from 15 to 60 minutes.

Graphical analysis from Fig. 8 suggested that HA proportion in biphasic mixture of HA and β-TCP increased steadily when reaction time was increased from 15 to 60 minutes in case of calcined MgHA powder synthesized without TEA. However, for calcined MgHA synthesized with TEA assistance, HA proportion increased with fluctuating rate. Thus after 60 min. of reaction of sol, HA proportion increased to 0.38 and 0.36 in calcined MgHA nanopowders, synthesized with TEA assistance and without TEA, respectively. This suggested that presence of TEA promoted MgHA synthesis. Thus, the 2-way ANOVA results suggested that reaction time had considerable impact on the proportion of HA and TEA assistance promoted MgHA synthesis.

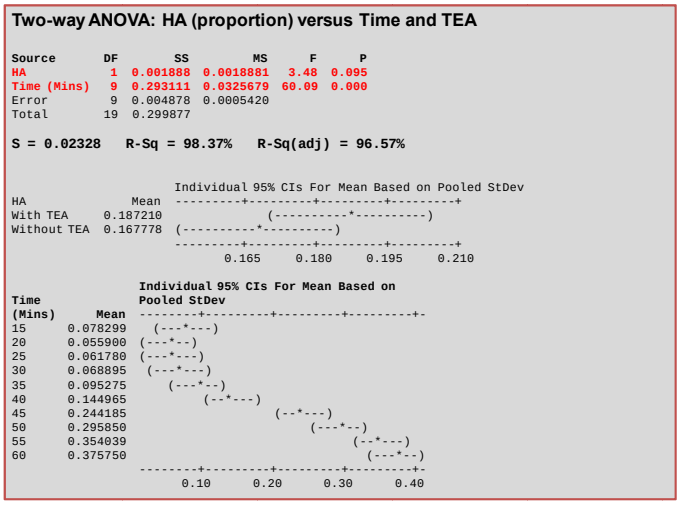


(a)



(b)

Fig. 6 XRD pattern of calcined MgHA powders (a) with TEA and (b) without TEA



Two-way ANOVA: HA (proportion) versus Time and TEA

Source	DF	SS	MS	F	P
HA	1	0.001888	0.0018881	3.48	0.095
Time (Mins)	9	0.293111	0.0325679	60.09	0.000
Error	9	0.004878	0.0005420		
Total	19	0.299877			

S = 0.02328 R-Sq = 98.37% R-Sq(adj) = 96.57%

Individual 95% CIs For Mean Based on Pooled StDev

HA	Mean
With TEA	0.187210
Without TEA	0.167778

0.165 0.180 0.195 0.210

Individual 95% CIs For Mean Based on Pooled StDev

Time (Mins)	Mean
15	0.078299
20	0.055900
25	0.061780
30	0.068895
35	0.095275
40	0.144965
45	0.244185
50	0.295850
55	0.354039
60	0.375750

0.10 0.20 0.30 0.40

Fig. 7 Two way ANOVA test results to analyse the effect of reaction time and TEA on the HA proportion in biphasic mixture of HA and β-TCP for reaction time from 15 to 60 min

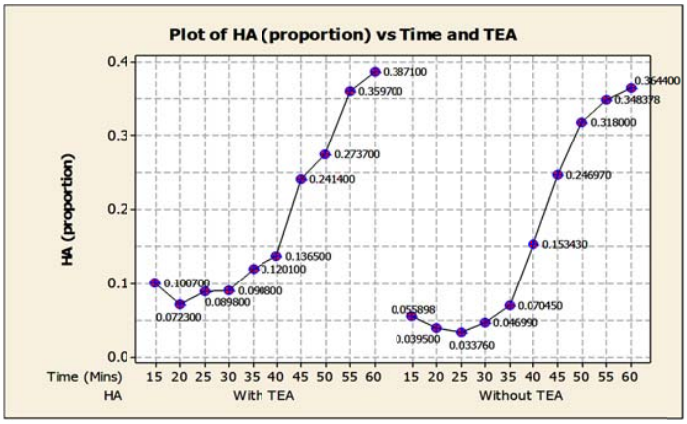


Fig. 8 Mean level of HA proportion for processes with TEA and without TEA

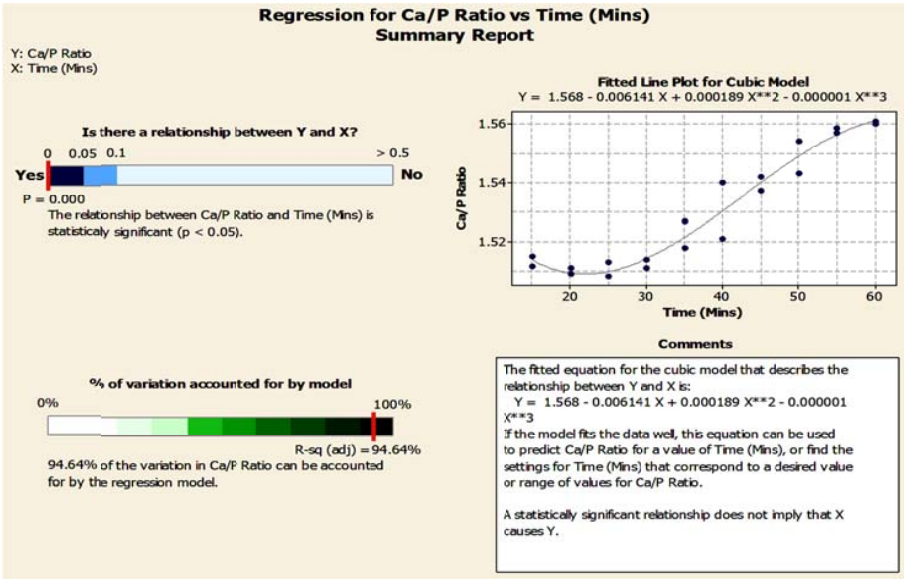


Fig. 9 Regression analysis

D. Variation of Ca/P ratio

The data collected for Ca/P ratio was calculated using (5) reaction time from 15 to 60 min. To investigate the relationship between Ca/P ratio and reaction time orthogonal regression tool was used. The correlation graphically presented in Fig. 9 was estimated and p and R^2 were found and results obtained were significant as $p < 0.05$ and $R^2 = 0.946$. The fitting equation (5) correlated Ca/P ratio with reaction time:

$$\frac{Ca}{P} 1.568 + 0.006141\, t - 0.000189\, t^2 - 0.000001\, t^3 \text{m} \qquad (5)$$

where t is the reaction time.

IV. CONCLUSIONS

The effect of reaction time and assistance of capping agent like TEA during the sol-gel synthesis of magnesium doped hydroxyapatite powder has been studied on proportion of hydroxyapatite phase and Ca/P ratio and the crystallite size. The magnesium doping occurred in HA as early asat a reaction time of 15 minutes. There were 90% chances to obtain MgHA powder during sol-gel synthesis by TEA assistance with crystallite size lower by 14 nm than that without TEA. The reaction time has significant influence on the HA phase proportion in the biphasic mixture of Ha and β-TCP and Ca/P ratio of synthesized powder while TEA has trivial impact. The Ca/P ratio had cubic relation with reaction time given by equation $Ca/P = 1.568 + 0.006141\ t - 0.000189\ t^2 - 0.000001\ t^3$. Only 58.3% of sol-gel synthesis of MgHA nanopowder could be completed in 60 min. of reaction time in present investigation.

REFERENCES

[1] L. B. Hossein-Nezhad, A. Maghbooli, Z. Bandarian, F. Mortaz, S. Soltani, Association of Bone Mineral Density and Lifestyle in Men, *Iran. J Publ Heal.* Vol. 35 2007, pp. 51–56.

[2] M. H. Fathi and A. Hanifi "Evaluation and characterization of nanostructure hydroxyapatite powder prepared by simple sol-gel method," *Materials Letters*, 2007, vol.61, pp. 3978-3983.

[3] M. M. Savalani, L. Hao, P. M. Dickens, Y. Zhang, K. E. Tanner and R. A. Harris, "The effects and interactions of fabrication parameters on the properties of selective laser sintered hydroxyapatite polyamide composite biomaterials," *Rapid Prototyping Journal*, 2012, vol.18, 1, pp.16-27.

[4] M. Percival, "Bone Health & Osteoporosis", *Appl. Nutr. Sci*. Rep., 1995, vol.5, pp. 1-5.

[5] T. Ioanovici, "Influence of magnesium doping on synthesized hydroxyapatite using the wet precipitation method", E-Health and Bioengineering Conference (EHB), 2011, pp. 1-4.

[6] R. Ramachandra, H. N. Roopa, T. S. Kannan, "Solid state synthesis and thermal stability of HAP and HAP-β-TCP composite ceramic powders". *J Mater Sci Mater Med* 1997, vol.8, pp. 511-518.

[7] N. Pramanik, A. Tarafdar, and P. Pramanik, "Capping agent assisted synthesis of nano sized hydroxyapatite comparative studies of the physicochemical properties," *Journal of materials processing technology*, 2007, vol.184, pp. 131-138.

[8] M.H. Fathi, A. Hanifi, and V. Mortazavi, Preparation and Bioactivity Evaluation of Bone-Like Hydroxyapatite Nano-powder, *J. Mater. Process. Technol*., 2008, vol.202, pp. 536–542.

[9] R. Jenkins and R.L. Snyder, *Introduction to X-ray Powder Diffractrometry*, Wiley, New York, 1996.

[10] S.V. Dorozhkin, Mechanism of solid-state conversion of non-stoichiometric hydroxyapatite to biphasic calcium phosphate: *Russ Chem Bull Int,* 2003, 52, pp. 2369-2375.

[11] B.D. Cullity and S.R. Stock, *Elements of X-Ray Diffraction*, 3rd ed., Prentice-Hall Inc., Englewood Cliffs, 2001.

[12] S. Kannan, J.M. Ventura, and J.M.F. Ferreira, Aqueous Precip- itation Method for the Formation of Mg-Stabilized b-Tricalcium phosphate: An X-ray Diffraction Study, *Ceram. Int.,* 2007, vol.33, pp. 637–641

[13] I. V Fadeev, L. I. Shvorneva, S. M. Barinov, and V. P. Orlovskii, "Synthesis and Structure of Magnesium-Substituted Hydroxyapatite," *Inorg. Mater.,* 2003, vol.39, pp. 947–950

[14] S. C. Afshar A, Ghorbani M, Ehsani N, Saeri MR, "Some important factors in the wet precipitation process of hydroxyapatite," *Mater Des*. 24 (n.d.) pp.197–202.

[15] H. K. Varma, S. S. Babu, "Synthesis of calcium phosphate bioceramics by citrate gel pyrolysis method," *Ceram Int,* 2005, vol.31, pp. 109-114.

[16] Z. Aizawa, H. Ueno, K. Itatani, Synthesis of calcium-deficient apatite fibers by a homogenous precipitation method and their characterization, *J Eur Ceram Soc., 2006,* vol.26, pp. 501–507.

[17] J. H. Park, D. Y. Lee, K. T. Oh, and Y. K. Lee, "Bioactivity of calcium phosphate coatings prepared by electrodeposition in a modified simulated body fluid," *Materials Letters*, 2006, vol.60, pp. 2573-2577.

[18] U. Vijayalakshmi, S. Rajeswari, "Preparation and characterization of microcrystalline hydroxyapatite using sol-gel method," *Trends Mater Artif Org,* 2006, vol.19, pp. 57-62.

[19] B. J. Singh, and D. Khanduja, "Perspectives of Control Phase to manage Six Sigma implements: A Foundry Case", *International Journal of Business Excellence*, 2014, vol.7, 1, pp. 88-111.