Effective role of CaO/P2O5 ratio on SiO2-CaO-P2O5 glass system

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In the present work, the effect of the CaO/P2O5 ratio on the composition of sol-gel synthesized 58SiO2-(19 – x)P2O5–(23 + x)CaO (x = 0, 5, 10 and 15 mol%) glass samples was studied. Further, the effect of NBO/BO ratio on hydroxy carbonated apatite layer (HCA) forming ability based on dissolution behavior in simulated body fluid (SBF) solution was also investigated. CaO/P2O5 ratios of synthesized glasses samples were 1.2, 2.36, and 9.5, respectively. NBO/BO ratios were obtained using Raman spectroscopic analysis as 0.58, 1.20, 1.46, and 1.78, respectively. All samples were soaked in the SBF solution for 7 days. The calculated weight losses of these samples were 58%, 64%, 83%, and 89% for corresponding NBO/BO ratios. The increase in CaO/P2O5 ratio increases the NBO/BO ratios. However, the increase in NBO/BO ratio increases HCA forming ability of SBF treated samples. The HCA crystalline layer formation was confirmed through X-ray Diffraction (XRD), Transmission Electron Microscopy (TEM), Scanning Electron Microscopy (SEM), Raman and Infrared spectroscopic analysis. Higher CaO/P2O5 ratio favors the increase in HCA formation for SBF treated calcium phosphosilicate glasses.

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Introduction

SiO2-CaO-P2O5 based glasses constitute a promising material for bioactive applications for bone repair, tissue regeneration in the human body, etc. [1]. Implantation of these materials in the human body induces a specific biological response at the material interface and can promote new bone formation without forming fibrous tissues. This new bone can form a bond to living bone inside the human body [2]. The bone bonding ability of these materials has been attributed to the deposition and growth of a hydroxyapatite (HA) layer, which is close to bone mineral composition [3]. In crystallization process, HA layer can get converted as hydroxy carbonated apatite (HCA) layer in the presence of SBF solution [4].

Sol-gel technique is an alternative route to synthesize the bioactive glasses with higher purity and homogeneity in comparison with conventional melt quenching technique [5–8]. Compared to the melt quenching method, sol-gel method enables obtaining the glasses with higher porosity and surface area to improve bone bonding rates and excellent resorption and degradation in physiological environments [2,9,10]. The limitation of SiO2 content to get HA layer for SBF soaked calcium phosphosilicate glasses is 60 mol% in melt quenching method and 90 mol% in sol-gel method. Due to this reason, the sol-gel method is the best feasible technique to get a HA layer formation compared to melt quenching method [11–14].

HCA layer formation in the presence of SBF solution for glasses depends on different process parameters such as glass composition [15], porosity [11], preparation method [16], precursors [6], and sintering temperature [17]. In bio-medical applications, HCA formation in SBF solution mainly depends on the dissolution behavior of the glass matrix [18]. In dissolution process, glass network connectivity is one of the interesting factors [6]. In the case of calcium phosphosilicate glasses, SiO2 and P2O5 are network formers. The commonly used network modifiers such as CaO and Na2O release cations of Ca2+, Na+ which migrate into SBF solution. This process eventually leads to the disconnectivity of the glass network and results in the formation of silanol groups. Later, it can affect the formation of silica gel layer through the polycondensation process, which acts as an implanted material for HCA formation [19].

In the case of CaO-P2O5-SiO2 gels, increase in SiO2 content increases the crystalline intensities of β and γ-(Ca(PO3)2) phases [20]. Laczkà et al. [21] reported that gel polymerization and crystallization process at different temperature conditions depend on the selection of precursors for CaO and P2O5 contents. Sopak et al. [22] reported the precipitation mechanism for CaO-SiO2-P2O5 glasses.
system depends on different Ca/P ratios at different pH values, and also revealed that increase in calcium content increases amorphous nature.

For SiO2–CaO glasses HCA forming ability in SBF solution depends on the ratio of sample weight to volume of SBF solution in incubation conditions [5]. For SiO2–CaO–P2O5 glasses, the studies related to the improvement in the HCA growth rate in SBF solution are available based on precursors used in the synthesis process and heat-treatment conditions [2]. According to Ahsan and Mortuza [23], the addition of P2O5 up to 5 mol% can depolymerize the glass system. In calcium phosphosilicate glasses, orthophosphate units de-polymerize the glass system and can also play the same role as Na2O, i.e., network modifier [21]. Sun et al. [24] reported that the increase in P2O5 composition (P2O5 > 9%) can enhance the degree of polymerization by acting as a network former [25].

In this work, 58SiO2–(19 – x)P2O5–(23 + x)CaO [x = 0, 5, 10 and 15 mol%] glasses have been synthesized using the sol-gel method. These glasses were soaked in the SBF solution for 7 days to get HCA formation on the glass surface. Thermal, structural and morphological properties were studied using X-ray Diffraction (XRD) technique, Thermo Gravimetric Analysis with Differential Thermal Analysis (TGA/DTA) and Scanning Electron Microscopy with Energy Dispersive X-ray (SEM/EDX) Analysis. Raman, Fourier Transmission Infrared (FTIR), and Transmission Electron Microscopy with Selected Area Energy Dispersive (TEM/SAED) techniques were performed on these glasses. Notably, the NBO/BO ratio effect on HCA forming ability studies for SiO2–CaO–P2O5 bioactive glass system in SBF solution, is not adequate. In the present study, NBO/BO ratio was found using Raman spectroscopic analysis. The impact of CaO/P2O5 content on NBO/BO ratio and the effect of NBO/BO ratio on HCA forming ability for SBF soaked glass samples were studied in detail.

Experimental

58SiO2–(19 – x)P2O5–(23 + x)CaO [x = 0, 5, 10 and 15 mol%] glasses were synthesized by conventional sol-gel process and samples were named as SCP1, SCP2, SCP3, and SCP4, respectively, as shown in Table 1. Chemicals for synthesis were purchased from Merck company (Mumbai, India). The precursors used for the preparation of these glasses were tetraethylorthosilicate [Si(OCH2CH3)4], triethylphosphate (TEP) [(C2H5O)3PO], calcium nitrate tetrahydrate [Ca(NO3)2-4H2O]. Further, H2O, 2 N of HNO3 were selected as solvents [(mol of H2O)/(mol of TEOS + mol of TEP) = 10] and [(mol of HNO3)/(mol of TEOS + mol of TEP) = 0.05], respectively. Tetraethylorthosilicate (TEOS) was mixed with H2O, HNO3 and stirred for one hour. At an interval of one-hour TEP, calcium nitrate was added subsequently and the solution was stirred well. The prepared sols were poured into Teflon beakers, sealed with parafilm and stirred for one hour. At an interval of one hour TEP, calcium nitrate was added subsequently and the solution was stirred well. The prepared sols were poured into Teflon beakers, sealed with parafilm and stirred for one hour. At an interval of one hour TEP, calcium nitrate was added subsequently and the solution was stirred well. The prepared sols were poured into Teflon beakers, sealed with parafilm and stirred for one hour. At an interval of one hour TEP, calcium nitrate was added subsequently and the solution was stirred well. The prepared sols were poured into Teflon beakers, sealed with parafilm and stirred for one hour. At an interval of one hour TEP, calcium nitrate was added subsequently and the solution was stirred well.

The SBF solution was prepared by dissolving KH2PO4, CaCl2, NaHCO3, MgCl2·6H2O, KCl and NaCl in deionized water (at pH = 7.4) with Tris-buffer, by maintaining the temperature at 37 °C [1]. The pelletized SCP samples were soaked in SBF solution on the basis of sample surface area/SBF solution volume ratio as 8 mm2/mL.

Characterization

The glass transition temperature (Tg) and onset crystalline temperature (Tx) were identified by the TGA and DTA analysis (SII EXTRAR 6000, Japan) with a flow rate of 10 °C/min in the temperature range 27–1000 °C. Weight loss of samples, before and after SBF treatment was measured using an electronic weighing balance [Sartorius, BT224s, India]. The structural properties of all samples have been investigated using the Powder X-ray Diffractometer (Rigaku, Minipuls 600, Japan) with a scan rate of 2°/min.

Spherical shaped HA crystalline nuclei of SCP samples were observed by TEM/SAED (JEOL JEM 2100, Japan), SEM (JEOL JSM-6380LA, Japan) and elements present in the samples were identified by the EDX analyzer (JEOL, Japan). The types of chemical bands were identified by the FTIR spectrometer (SHIMADZU-8400s, North America). For FTIR analysis, the pellets were prepared using 300 mg of KBr mixed with 1 mg quantity of stabilized and SBF treated SCP glasses. The pellets were analyzed in the wave number range between 400 and 1800 cm⁻¹ at a rate of 25 scans/min with the resolution of 4 cm⁻¹. Room temperature Raman spectroscopy was performed using a LABRAM-HR8000 (Japan). To avoid laser heating of the samples, the incident power was kept at a low value of 2 mW. The pH value of SBF solution was measured using pH meter (Eutech, pH 510, India) before and after soaking SCP samples. Ca²⁺ and PO4³⁻ ion concentrations were measured using Flame Photometer (ELICO CL378, Germany) and UV/Vis absorption spectrometer (HITACHI PM & E 101, Canada).

Results and discussion

TGA/DTA analysis

Thermal behavior SCP samples were studied using TGA/DTA analysis and the results are shown in Fig. 1(a–d). Two weight losses (TWL1 and TWL2) were observed for SCP samples at different temperature conditions using TGA curves. The first weight loss (WL1) was observed at 452–494 °C related to the evaporation of organics (alkoxy groups) [26–27]. The second weight loss (WL2) related to the thermal evaporation of residual nitrates has been observed at 545–563 °C [26–28]. Glass transition (Tg) temperature and crystalline onset temperature (Tx) values were measured three times using the DTA curves for SCP dried gels as shown in Table 1. The glassy forming ability is naturally related to the crystalline phase itself. The variations in Tg and Tx values are related to the change of the primary crystalline phase.

Lucas-Girot et al. [29] and Letaief et al. [30] reported that for low P2O5 content, phosphorous is not considered as a glass former like silicon and it is present in the glass structure as PO4³⁻ ions like a glass modifier. Aguiar et al. [31] observed that, to get HA formation for SiO2–P2O5–CaO–Na2O–MgO glasses, phosphorus does not act as

| x mol% | Glass sample name | SiO2 (mol%) | P2O5 (mol%) | CaO (mol%) | First weight loss (°C) | Second weight loss (°C) | Tg (°C) | Tx (°C) |
|--------|------------------|-------------|-------------|------------|---------------------|----------------------|---------|--------|
| 0      | SCP1             | 58          | 19          | 23         | 452                 | 554                  | 402 ± 0.19 | 723 ± 0.20 |
| 5      | SCP2             | 58          | 14          | 28         | 488                 | 545                  | 479 ± 0.28 | 764 ± 0.13  |
| 10     | SCP3             | 58          | 9           | 33         | 494                 | 558                  | 415 ± 0.07 | 726 ± 0.09  |
| 15     | SCP4             | 58          | 4           | 38         | 494                 | 563                  | 481 ± 0.15 | 774 ± 0.15  |
Silicate glasses enhance the bioactivity with inclusion of a small P$_2$O$_5$ amount. This remarkable inversion in the effect of P$_2$O$_5$ would be explained in the following way. Some of the phosphorous forms P-O-Si links and reduces the bioactivity (considered as negative effect) and some other is found as free orthophosphate, whose relatively fast initial release accelerates the HA deposition and boosts the bioactive process (considered as positive effect). The balance between these opposite effects decides the bioactivity of the P-containing composition. Based on the bioactivity data of the compositions modeled, Tilocca and Cormack [32] concluded that the negative effect prevails for high P$_2$O$_5$ fractions, whereas positive effect prevails for that lower (below 10 mol% P$_2$O$_5$) fractions. From these literature supports, it could be concluded that P$_2$O$_5$ acts as a network former for SCP1 and SCP2 samples in the present study (with >10 mol% P$_2$O$_5$). For SCP3 and SCP4 samples P$_2$O$_5$ acts as a network modifier (with <10 mol% P$_2$O$_5$).

T$_g$ and T$_x$ values vary with P$_2$O$_5$ content. As the (x) mol% increases from 0 to 5%, the T$_g$ and T$_x$ values increase. The observed P$_2$O$_5$ quantity is greater than 10 mol% in SCP1 and SCP2 samples and in this case, it (P$_2$O$_5$) acts as network former. P$_2$O$_5$ content is higher for SCP1 compared to SCP2 sample. Network former addition polymerizes the silicate network and decreases the required temperature to get glass formation as reported in the literature [24,28]. As the P$_2$O$_5$ content is changing from 5 to 9 mol%, it acts as a network modifier. Compared to SCP2, SCP3 sample has less polymerization effect. In the network modifier case polymerization reduces and it leads to decrease in T$_g$ and T$_x$ values. As the mol% increases from 10 to 15% the T$_g$ and T$_x$ values increase. SCP3 and SCP4 samples have P$_2$O$_5$ less than 10 mol%. In this case, P$_2$O$_5$ acts as a network modifier and changes in T$_g$ and T$_x$ values depending on network modifier concentrations. Carta et al. [7] reported that increase in network modifier content increases T$_g$ and T$_x$ values for soda lime phosphosilicate glasses. CaO (network modifier) content is more in SCP4 than SCP3 sample and P$_2$O$_5$ also acts as a network modifier for these samples. Depending on the network modifiers, increase in glass transition temperature and crystalline onset temperature takes place [25]. There is no significant weight loss above 700°C in TGA curves. DTA curves show exothermic peaks above 700°C. Due to this reason, 700°C is considered as a stabilization temperature for SCP samples [27,28,33].

**XRD analysis**

The XRD pattern of SCP samples is shown in Fig. 2(a). The XRD pattern has broadband between the diffracted angles 20°–30°, indicating the amorphous nature. This occurs due to the internal disorders and glassy nature of the materials sintered at 700°C. It was also confirmed by DTA analysis. The SBF treated samples show the crystalline nature [as shown in Fig. 2(b)]. The dominant HA crystallite peak was identified at 2θ = 32° [(h k l) = (211)] corresponding to Ca$_5$(PO$_4$)$_3$(OH) [HA] (JCPDS 01-074-0565). Calcite phase also was observed at 2θ = 29° (JCPDS NO 01-081-2027) [27,34]. Another HA peak was observed at 38°. The intensities of three major reflections (211), (300), and (002) increased from SCP1 to SCP4 sample.

During SBF treatment, a chemical reaction takes place on the sample surface. In this process calcium, phosphate ions migrate into SBF solution and form silanol groups. Due to polycondensation process silica gel layer forms on the sample surface. Calcium and phosphate ions migrate through silica gel layer and form calcium phosphate (apatite) layer on the sample surface. Due to crystallization process between apatite layer and existed calcium, phosphate, hydroxyl ions in SBF solution, hydroxyapatite (HA) layer forms on the sample surface [6,11]. HA layer growth depends on Ca$^{2+}$ and PO$_4^{3-}$ ion dissolution of the sample. Lower P$_2$O$_5$ content favors the formation of HA.
formation of more orthophosphate (PO$_4^{3-}$) units in the sample. Increase in CaO and decrease in P$_2$O$_5$ contents increase the Ca$^{2+}$ and PO$_4^{3-}$ ions which are released into SBF solution. This further leads to increase in HA layer formation on the sample surface [33]. Due to this reason, the HA crystalline intensities have increased from SCP1 to SCP4 sample. CaO/P$_2$O$_5$ ratios for SCP1 to SCP4 samples are 1.2, 2.3, 6.9, and 9.5, respectively. From these observations, it can be confirmed that increase in CaO/P$_2$O$_5$ ratio increases the HA crystallinity for SBF treated samples.

HA formation is increased from SCP1 to SCP4 sample. Formed HA consumes Ca$^{2+}$ ions and getting released into SBF solution, it leads to decrease in Ca$^{2+}$ ion concentration in SBF solution and forms CaCO$_3$ layer due to a chemical reaction between calcium and carbonate ions [18]. Due to this reason, calcite crystalline peak intensities have decreased from SCP1 to SCP2 samples (In this case P$_2$O$_5$ acts as network former).

The calcite intensities were increased from SCP2 to SCP3 sample, since the polymerization effect is more in SCP2 than SCP3 sample (in the case of SCP3, P$_2$O$_5$ acts as network modifier). The number of Ca$^{2+}$ ions released by SCP2 samples is less and these Ca$^{2+}$ ions react with PO$_4^{3-}$ ions and form HA layer. Dissolution of Ca$^{2+}$ and PO$_4^{3-}$ ions is more in SCP3 sample and forms HA layer with less Ca$^{2+}$ ions expense of SBF. Calcite intensities were decreased from SCP3 to SCP4 sample, since formed HA consumes more Ca$^{2+}$ ions.

**Surface morphology**

The surface morphology of SCP samples before and after SBF treatment is shown in Fig. 3. For SBF untreated SCP samples EDX analysis confirmed that elements present in the samples are Si, Ca, P and O as shown in Fig. 3(a, e, i and m). SBF untreated samples have a homogeneous surface morphology [Fig. 3(b, f, j and n)]. For SBF treated samples, the surface morphology [Fig. 3(c, g, k and o)] clearly exhibits the spherical shaped HCA nuclei formation on the sample surface. It is observed that the lower P$_2$O$_5$ concentration (increase in CaO) leads to increase in HCA nuclei progressively on the glass surface. For SBF treated samples, the EDX analysis of HCA layer has shown the presence of Ca, P, C and O elements on the sample surface [Fig. 3(d, h, l and p)]. The increase in Ca and P intensities of these samples indicates the increase in HA layer formation on the sample surface. In the previous section, it has been discussed that the crystallization process between apatite and existed calcium, phosphate and hydroxyl ions leads to HA formation on the sample surface. In this process, with CO$_3^{2-}$ presence, HA layer gets converted into as HCA layer [6].

All SBF treated samples show good homogeneity in particle size with the relevant HCA particle size distributions. HCA nuclei average sizes were increased for SCP1 to SCP4 samples as from 821 nm to 1259 nm. It indicates that the average particle sizes of HCA nuclei have increased with increase in CaO/P$_2$O$_5$ ratio, and similar studies have been reported in the literature. SCP samples consist of CaO content in the increasing order from SCP1 to SCP4 samples. The increase in CaO content increases Ca$^{2+}$ ions release into SBF solution and it leads to increase in HA layer formation on the sample surface. EDX analysis shows that Ca and P intensities [related to HA] increased from SCP1 to SCP4 sample.

**Raman analysis**

For SCP samples Si-O-Si asymmetric stretching (as s) and Si-O-NBO asymmetric stretching (as s) modes were assigned at 1027–1080 cm$^{-1}$ and 961–967 cm$^{-1}$ wave number regions, respectively, as shown in Fig. 4(a) [Table 2]. Fivefold symmetric stretching [W$_1$] modes were also observed at 460–477 cm$^{-1}$. For the identification of NBO modes in silicate tetrahedra, the deconvolution process has been used in the wave number range of 800–1200 cm$^{-1}$ [Fig. 4(b–e)]. Si-O-NBO (as s) intensity/Si-O-BO (as s) intensity [NBO/BO] ratios have been obtained using deconvolution process. The deconvolution curve fittings were considered based on the fitting curve area, proportional to band intensities [36]. The obtained NBO/BO ratios of SCP1, SCP2, SCP3 and SCP4 samples are 0.58, 1.20, 1.46, and 1.78, respectively. Increase in CaO/P$_2$O$_5$ ratio increases NBO/BO ratio and increase in NBO/BO ratio decreases the degree of polymerization between silicate and phosphate tetrahedra. The electronegativity of Si$^{4+}$ is predominated by the electronegativity of P$^{5+}$ ion for SCP1 and SCP2 glasses. Due to this, NBOs of silicate tetrahedra convert as that of phosphate tetrahedra. Repolymerization takes place between silicate and phosphate tetrahedra. Decrease in P$_2$O$_5$ decreases the polymerization, and it leads to increase in the T$_g$, T$_x$ values from SCP1 to SCP2 glass. SCP3 and SCP4 glasses have less P$_2$O$_5$ compared to SCP1 and SCP2 glasses, and in this case electronegativity of Si$^{4+}$ predominates the electronegativity of P$^{5+}$ ion. The NBO conversion from silica tetrahedra to phosphate tetrahedra is very less for SCP3 compared to SCP2 sample, and in this case phosphate phases form as clusters. Compared to SCP1, in SCP3 sample Phosphate phase size (cluster size) is more. O’Donnell et al. [37] reported that increase in Phosphate phase size decreases the T$_g$ and T$_x$ values. Due to this reason T$_g$ and T$_x$ values were decreased from SCP2 to SCP3 glass. In the case of SCP3 and SCP4 samples, decrease in P$_2$O$_5$ content decreases the phosphate phase size. SCP4 sample has less P$_2$O$_5$ compared to SCP3 sample, and due to this reason phosphate phase size decreases in SCP4 compared to SCP3 sample. Ca$^{2+}$ ion effect is also more for...
SCP4 compared to SCP3 sample, and it causes the increase in $T_\beta$ and $T_x$ values for SCP4 compared to SCP3 sample.

For SBF treated samples $CO_3^2-$ asymmetric stretching modes appeared in the wave number range of 1084–1086 cm$^{-1}$ as shown in Raman spectra [Fig. 4(f)]. $PO_4^{3-}$ symmetric stretching modes were also observed at 954–965 cm$^{-1}$ wave number region. For SCP4 samples HA related crystalline $PO_4^{3-}$ bending modes and fivefold symmetric stretching [W1] modes were also observed at 591 cm$^{-1}$ and 433 cm$^{-1}$, respectively [Table 2]. Crystalline intensities of $CO_3^2-$ modes increased from SCP1 to SCP4 sample. Due to this reason HCA formation also increases for SCP samples, and also that the HCA growth is carbonates depended [38].

FTIR analysis

FTIR spectroscopic analysis of SCP samples is shown in Fig. 5(a). The transition bands observed at 1064–1187 cm$^{-1}$ were assigned to the Si-O-Si asymmetric stretching (as s) and Si-O-NBO (as s) modes were assigned at 1026–1033 cm$^{-1}$. For SCP samples, different intensities occur at 466–478 cm$^{-1}$ related to rocking modes of Si-O-Si [Table 3]. Si-O-Si bending modes were observed in the wave number region of 779–794 cm$^{-1}$. $PO_4^{3-}$ asymmetric stretching modes of vibrations are also observed at 1222–1265 cm$^{-1}$. The decrease in $P_2O_5$ content decreases the degree of polymerization between silicate and phosphate tetrahedra, and due to this reason phosphate tetrahedra have less prominence and silicate tetrahedra have more prominence supported by the literature [33].

For SBF treated samples [Fig. 5(b)] Si-O-Si asymmetric stretching mode became broader compared to SBF untreated samples in the FTIR spectra (1022–1087 cm$^{-1}$), and this is due to the formation of silica gel (amorphous) layer on the sample surface in the dissolution process. For SBF treated samples $CO_3^2-$ bending (1413–1498 cm$^{-1}$) modes became broader and more prominent compared to SBF untreated samples. Sharp, intense $CO_3^2-$ stretching modes (~873 cm$^{-1}$) were also observed for SBF treated samples compared to SBF untreated samples [Table 3]. For SBF treated samples $PO_4^{3-}$-bending amorphous mode (601–605 cm$^{-1}$) broadness was decreased and sharpness was increased compared to SBF untreated.
samples. The increase in sharpness is related to the formation of PO₄³⁻ bending crystalline modes which are assigned at 669 cm⁻¹.

From these observations, it can be concluded that during the crystallization process the HCA crystalline layer would be formed in the presence of carbonate groups. XRD, SEM/EDX and Raman spectroscopic analysis confirmed the HCA crystal formation on the sample surface in dissolution process.

It is also observed that the OH groups are also present in the FTIR spectra before and after SBF treatment at 1635–1643 cm⁻¹ and 1641 cm⁻¹ wave number regions, respectively. It may be due

Table 2
Raman band assignments of Calcium phosphosilicate glasses before and after soaking in SBF solution [38–41].

| Raman absorption band in cm⁻¹ | SCP1 | SCP2 | SCP3 | SCP4 | Assigned bands | SCP1 | SCP2 | SCP3 | SCP4 | Assigned bands |
|-------------------------------|------|------|------|------|----------------|------|------|------|------|----------------|
| 463                           | 471  | 477  | 460  | –    | W1             | –    | –    |–     | 433  | W1             |
| 961                           | 967  | 966  | 965  | –    | Si-O-NBO asymmetric stretching | –    | –    |–     | 591  | PO₄³⁻ Modes of HA |
| 1073                          | 1027 | 1080 | 1076 | –    | Si-O-Si asymmetric stretching | 954  | 954  | 963  | 965  | PO₄³⁻ symmetric stretching |
|                               | –    | –    |–     | 1050 | Si-OH          | –    | –    | 1084 | 1084 | CO₃²⁻ stretching |

Fig. 4. (a) Raman spectra of SCP samples before SBF treatment, (b), (c), (d) and (e) are deconvoluted Raman spectra of SCP1, SCP2, SCP3 and SCP4 samples respectively. (f) Raman spectra of SCP samples after SBF treatment.
to absorbed water from the environment during the pelletization process [39]. Si-O-Si rocking, bending modes were present in the wave number range of 464–470 cm⁻¹ and 785–794 cm⁻¹, respectively after SBF treatment. Non-Bridging oxygen modes related to Si-O-Ca were also observed for SBF treated SCP3 and SCP4 samples at 923–925 cm⁻¹.

**TEM/SAED analysis**

The TEM analysis revealed that SBF treated samples have spherical shaped particles as shown in Fig. 5(c–f). The d-spaces [for (211) plane] for spherical shaped particles are found using TEM/SAED pattern [40]. The observed d-spaces for SCP1, SCP2, SCP3, and SCP4 samples are 0.280 nm, 0.283 nm, 0.279 nm, and 0.281 nm, respectively. The observed d_{(211)}-spaces are in good agreement with standard JCPDS (24-0033) files of HCA layer. From these observations, it can be concluded that for SBF treated samples the formed crystals are HCA particles in the dissolution process.

**pH assessment, dissolution and weight loss studies**

In dissolution process, calcium and phosphate ions migrate into SBF solution, forming silanol groups on the sample surface. In polycondensation process silanol groups form silica gel layer on the sample surface. Calcium and phosphate ions of glass matrix leach on the silica gel layer surface as amorphous calcium phosphate (apatite) layer, and it changes the Ca²⁺ and PO₄³⁻ concentrations of SBF solution. Incorporation of Ca²⁺ and PO₄³⁻, and hydroxyl and

![Fig. 5. (a), (b) FTIR spectra of SCP samples before and after SBF treatment. (c), (d), (e), and (f) TEM/SAED analysis of SBF treated SCP1, SCP2, SCP3, and SCP4 samples.](image-url)
carbonate ions from SBF solution into apatite layer lead to HCA formation on the sample surface in the crystallization process [6].

The pH values are increased up to 24 h of soaking time in the SBF solution as shown in Fig. 6 (a). At this stage, due to the fast release of $\text{Ca}^{2+}$ ions silanol groups have formed and it leads to the HA formation on the sample surface [18,27]. The pH values are almost stabilized after 24 h. The pH values, $\text{Ca}^{2+}$ and $\text{PO}_4^{3-}$ ion concentrations of SBF solution are increased [as shown in Table 4] from SCP1 to SCP4 samples as shown in Fig. 6 (b) and (c). The weight loss of SBF treated SCP samples is also increased from SCP1 to SCP4 samples [Fig. 6(d)].

Raman and FTIR spectroscopy analysis of SCP samples revealed that the non-bridging oxygens exist as Si-O-Ca asymmetric stretching modes. The Raman spectroscopic analysis also revealed that NBO/BO ratio is increased for SCP samples with an increase in CaO/P$_2$O$_5$ ratio.

The $\text{Ca}^{2+}$ and $\text{PO}_4^{3-}$ ions release from glass matrix in the SBF solution depending on the $\text{Ca}^{2+}$ and $\text{PO}_4^{3-}$ ions in the glass matrix.
Table 4
| Soaking time in SBF (days) | PO₄³⁻ ion concentration (ppm) | Ca²⁺ ion concentration (ppm) |
|--------------------------|-------------------------------|-------------------------------|
|                          | SCP1 | SCP2 | SCP3 | SCP4 | SCP1 | SCP2 | SCP3 | SCP4 |
| 0                        | 100  | 100  | 100  | 100  | 100  | 100  | 100  | 100  |
| 3                        | 149.2| 152.8| 166.2| 168.9|
| 5                        | 165.6| 167.6| 175.1| 185.6|
| 7                        | 166.5| 168.2| 175.6| 187.5|

Table 5
| x mol% | NBO/BO ratio | Raman | Nuclei average size (nm) | SEM | d_(211) (nm) TEM/SAED | Weight loss for SBF soaked samples (%) |
|--------|--------------|-------|--------------------------|-----|-----------------------|--------------------------------------|
| 0      | 0.58         | 821   | 0.280                    |     | 74.5                  | 58                                   |
| 5      | 1.20         | 966   | 0.283                    |     | 78.5                  | 64                                   |
| 10     | 1.46         | 1176  | 0.279                    |     | 82.5                  | 83                                   |
| 15     | 1.78         | 1259  | 0.281                    |     | 86.5                  | 89                                   |

and degree of polymerization also. In the case of SCP1 and SCP2 samples, P₂O₅ acts as network former. Polymerization takes place between phosphate and silicate tetrahedra. P₂O₅ content is more and polymerization effect is also more for SCP1 than SCP2 sample, and due to this reason SCP2 sample releases more PO₄³⁻ ions than SCP1 sample. NBO/BO ratio is more for SCP2 sample than SCP1 sample and NBOs are in Si-O-Ca form. Due to low polymerization and higher NBOs, the Ca²⁺ release also increased from SCP1 to SCP2 sample. SCP3 and SCP4 samples have less P₂O₅ content, and in this case P₂O₅ acts as network modifier. Due to this reason polymerization effect is less for SCP3 and SCP4 samples, and orthophosphate units form as clusters with very weak P-O bonds [37]. Phosphate phase cluster size (with orthophosphate units) is less for SCP4 compared to SCP3 sample. Due to this reason, SCP4 glass can release more PO₄³⁻ ions than SCP3 sample. Due to increase in NBO/BO ratio, Ca²⁺ ion release also increases from SCP3 to SCP4 sample. From all these observations it can be concluded that the Ca²⁺ and PO₄³⁻ ion release from glass matrix in the SBF solution increases from SCP1 to SCP4 sample. The increase in NBO/BO ratio increases the dissolution of Ca²⁺ ions into SBF solution, and it causes the increase in pH values of the SBF solution in the dissolution process for SCP samples.

In dissolution process, HA layer formation on the sample surface not only depends on number of releasing Ca²⁺ and PO₄³⁻ ions from the sample, but also on the number of leaching Ca²⁺ and PO₄³⁻ ions from SBF solution. The increase in the Ca²⁺ ion release depends on an increase in NBO/BO ratio. The decrement in glassy nature is based on P₂O₅ content for SCP1 to SCP4 samples and it results in the increase in the dissolving PO₄³⁻ ions. The increase in weight loss of SBF treated samples [Table 5] in the dissolution process occurs from SCP1 to SCP4 samples based on the increment in number of dissolution of Ca²⁺ and PO₄³⁻ ions from the sample into SBF solution.

Conclusions

58SiO₂₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋ avalia-

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and degree of polymerization also. In the case of SCP1 and SCP2 samples, P₂O₅ acts as network former. Polymerization takes place between phosphate and silicate tetrahedra. P₂O₅ content is more and polymerization effect is also more for SCP1 than SCP2 sample, and due to this reason SCP2 sample releases more PO₄³⁻ ions than SCP1 sample. NBO/BO ratio is more for SCP2 sample than SCP1 sample and NBOs are in Si-O-Ca form. Due to low polymerization and higher NBOs, the Ca²⁺ release also increased from SCP1 to SCP2 sample. SCP3 and SCP4 samples have less P₂O₅ content, and in this case P₂O₅ acts as network modifier. Due to this reason polymerization effect is less for SCP3 and SCP4 samples, and orthophosphate units form as clusters with very weak P-O bands [37]. Phosphate phase cluster size (with orthophosphate units) is less for SCP4 compared to SCP3 sample. Due to this reason, SCP4 glass can release more PO₄³⁻ ions than SCP3 sample. Due to increase in NBO/BO ratio, Ca²⁺ ion release also increases from SCP3 to SCP4 sample. From all these observations it can be concluded that the Ca²⁺ and PO₄³⁻ ion release from glass matrix in the SBF solution increases from SCP1 to SCP4 sample. The increase in NBO/BO ratio increases the dissolution of Ca²⁺ ions into SBF solution, and it causes the increase in pH values of the SBF solution in the dissolution process for SCP samples.

In dissolution process, HA layer formation on the sample surface not only depends on number of releasing Ca²⁺ and PO₄³⁻ ions from the sample, but also on the number of leaching Ca²⁺ and PO₄³⁻ ions from SBF solution. The increase in the Ca²⁺ ion release depends on an increase in NBO/BO ratio. The decrement in glassy nature is based on P₂O₅ content for SCP1 to SCP4 samples and it results in the increase in the dissolving PO₄³⁻ ions. The increase in weight loss of SBF treated samples [Table 5] in the dissolution process occurs from SCP1 to SCP4 samples based on the increment in number of dissolution of Ca²⁺ and PO₄³⁻ ions from the sample into SBF solution.

Conclusions

58SiO₂₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋щения

Table 5

| x mol% | NBO/BO ratio | Raman | Nuclei average size (nm) | SEM | d_(211) (nm) TEM/SAED | Weight loss for SBF soaked samples (%) |
|--------|--------------|-------|--------------------------|-----|-----------------------|--------------------------------------|
| 0      | 0.58         | 821   | 0.280                    |     | 74.5                  | 58                                   |
| 5      | 1.20         | 966   | 0.283                    |     | 78.5                  | 64                                   |
| 10     | 1.46         | 1176  | 0.279                    |     | 82.5                  | 83                                   |
| 15     | 1.78         | 1259  | 0.281                    |     | 86.5                  | 89                                   |

The authors have declared no conflict of interest.

Compliance with Ethics Requirements

This article does not contain any studies with human or animal subjects.

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