



Development of Zirconia Substituted 1393 Bioactive Glass for Orthopaedic Application

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ABSTRACT

Zirconia was used as a bone substitute, since it has excellent mechanical properties. It is also good bioinert to bone tissue. In this study, we report on zirconia substituted 1393 bioactive glass. In novel melt-derived "1393" Bioactive glass (53 SiO₂, 6Na₂O, 12 K₂O, 5MgO, 20CaO, and 4 P₂O₅ wt %) with CaO was doped by ZrO₂ for and were melted at 1400°C in alumina crucibles in electric furnace with air as furnace atmosphere. Bioactivity of these samples were analysed by put in the SBF for different time periods. Hydroxy Carbonate Apatite layer was developed and identified by FTIR, XRD and SEM. Chemical durability was also determined by weight loss method. Density of the samples were determined and found to increase significantly with increasing amount of (0-2 wt %) zirconia, reason is due to replacement of lighter element of CaO has been replaced by heavier element ZrO₂. Finally, this investigation clearly concluded that ZrO₂ substituted bioactive glass would be potential biomaterials for biomedical applications.

Keywords: Bio-active glasses, Simulated body fluid, XRD, SEM, FTIR, pH, Chemical durability, Density.

INTRODUCTION

Ceramics which are used for filling and rebuilding of non-healing bone defects and for healing of damaged or diseased parts of the muscular-skeletal system is termed as bio ceramics¹. Bioinert (alumina, zirconia), bioactive (hydroxyapatite, glass, and glass-ceramics), resorbable (tricalcium phosphate) or porous for tissue in growth (hydroxyapatite-coated metals, alumina) may be used in the bio ceramic composites². Several types

of bioactive glasses are developed over past years. Bioactive glasses (BG) have been known for their bioactive properties and their aptitude to form a strong bond to bone by formation of hydroxyapatite surface layer³. Bioactive glasses (BGs) such as "45S5 Bio-glass[®]" (45 SiO₂, 24.5CaO, 24.5Na₂O, 6 P₂O₅ wt %) and "1393" (53 SiO₂, 6Na₂O, 12 K₂O, 5 MgO, 20 CaO, and 4 P₂O₅ wt %) compositions have been widely used for bone tissue engineering applications^{2,4}. Hench *et al.*, examined the in vitro bonding mechanism with synthetic material

because of the chemical reactions taking place over glass surface. These chemical reactions strongly help the implants to bond with the bone tissues; hence one can replace the diseased or damaged part of the human bone⁵. Many glass compositions have been developed by doping therapeutically active ions such as strontium, zinc, magnesium, fluoride and cobalt in silicate glass system have been discovered in the past⁶⁻⁹. Several researches are going on for preparation and characterization of glasses and glass-ceramics, doped with some ions such as Zr, Li, Fe, Ti, K, Zn, Sr and Mg because of their unique effect on differentiation, osteoblastic cell proliferation and thus bone mineralization¹⁰⁻¹⁵. The ZrO₂ substituted is widely used as a substrate in hard tissue applications due to its excellent strength and fracture toughness¹⁶. It was discovered from the reaction product obtained after heating gems by the German chemist Martin Heinrich Klaproth in 1789¹⁷. Several research articles have suggested that zirconia has good chemical and dimensional stability, mechanical strength and toughness and it is also biologically inert¹⁸. Many study have shown that the compressive strength of ZrO₂ is relatively higher than porous HA and ZA20 (20 wt. % Al₂O₃ added TZP) ceramics. *In vitro* evaluation has also shown that ZrO₂ is not cytotoxic¹⁹⁻²¹. Vyas *et al.*,²²⁻²⁷ in an earlier investigation had also shown that the addition of cobalt oxide and nickel oxide up to 0-2.0 wt% in 45S5 glass (BG) and glass-ceramic (BGC) has resulted an increase physico-mechanical properties and bioactivity of their samples due to formation Co-O-Si and Ni-O-Si bonds. Tripathi *et al.*,²⁸ have also found the characterization of Li₂O, CaO, Al₂O₃, P₂O₅, SiO₂ glasses as bioactive material and all the elastic moduli values were establish to increase with the increasing in Al₂O₃/Li₂O ratio.

In the current investigation an endeavour has been made to study the construct of zirconia doping in silica-based bioactive glass and to verify its bioactivity and other characteristics.

MATERIAL AND METHODS

Preparation of glass

For the source of SiO₂¹⁵, Na₂O, CaO, K₂O, P₂O₅ and MgO, Fine grained quartz, anhydrous sodium carbonate, anhydrous calcium carbonate, Potassium Carbonate, ammonium dihydrogen

orthophosphate and Magnesium Carbonate respectively were used⁴. The ZrO₂ is available as it is and was added for preparation of bioactive glasses. All the batch materials were of analytical grade chemicals and were used without further purification. The weighed batches were mixed properly with the help of mortar and pestle. Before mixing the mortar and pestle were cleaned thoroughly and allowed to dry properly. After thorough mixing of batch materials were kept in alumina crucible and placed in an electric furnace. The furnace temperature was set to 1400°C and the duration to reach 1400°C was set to 3 h and after reaching 140°C the steady state was maintained for more 3 hours. After melting the prepared bioactive glass samples were poured into moulds and were directly transferred to a regulated muffle furnace at the temperature of 500°C for annealing. The muffle furnace was left to cool to room temperature after 1 h Annealing is done to remove the internal stress after the glasses are formed.

Simulated Body Fluid (SBF)

Kokubo and his teams developed Simulated Body Fluid (SBF) which has similar inorganic ion concentrations to those of human extracellular fluid^{22, 29-31}. The ion concentrations of SBF are given on Table. 2.

Procedure

1. The HCl available in the laboratory is of 11.6 N. So it was diluted to 1 N-HCl by the following formula.

$$N_1 V_1 = N_2 V_2$$

Where N₁, N₂ = Normality of 1st and 2nd solution respectively

V₁, V₂ = Volume of 1st and 2nd solution respectively

We have taken 100 ml distilled water and we need to find out how much HCl of 11.6N is required to be mixed with it to get 1N-HCl solution.

$$11.6 \times V_1 = 1 \times 100 \\ \Rightarrow V_1 = 8.62 \text{ ml}$$

So 8.62 ml of 11.6N HCl was mixed with 100ml of distilled water to get 1N-HCl.

2. 700 ml of distilled water is poured into magnetic stirrer.

3. The 1 to 5 materials mentioned in the table 2 were added one by one.
4. The 1N-HCl was added to the above mixture of about 20 ml and pH was maintained to be 2.0/2.5.
5. Then the material numbered 7-8 were added one by one slowly.
6. Finally the tris-buffer was added very slowly. Then HCl was added and the pH was adjusted to be within 7.2/7.25.

Thus, SBF solution was prepared which was used for evaluation of bioactivity of artificial materials *in vitro*.

Characterization

Using the XRD, crystalline phases present in before and after SBF treated glass samples were identified. The samples were ground to 75 μ m and subjected to XRD using RIGAKU-Mini μ ex II diffractometer of range between 20 $^\circ$ and 80 $^\circ$. Fourier transform infrared (FT-IR) spectrometer (VARIAN scimitar 1000, USA) range was used to investigate the functional groups present in bio-glass samples in the range of 4000–400 cm $^{-1}$. The surface morphology of bioactive glass samples was analysed by SEM (Inspect S50, FEI) before and after SBF behaviour.

In vitro bioactivity test

In vitro test was performed to examine the bioactivity of all samples by immersing 0.5 g of each sample in 50 ml of SBF solution in a plastic box and incubated at 37.5 $^\circ$ C in a static condition for 2, 5, 7, 15 and 31 days. pH was also measured using Universal Biomicroprocessor pH meter calibrated with buffer solutions of pH 4.00 and 7.00 at room temperature. pH values have been recorded during above given time periods. The surface morphology of bioactive glass samples was analysed by SEM (Inspect S50, FEI) before and after SBF behaviour. The glass powders (1g) were hard-pressed (load of 10 MPa) into pellet forms in a hand pelletizer machine using an evocable die to produce discs of 10 mm in diameter. The carboxymethyl cellulose (CMC) was used as a binder for making pellets. The glass pellets were put in SBF (10 ml) for 15 days at 37.5 $^\circ$ C. Samples were coated with gold before SEM analysis.

Density measurement

The density of samples was measured by Archimedes principle at room temperature. For immersing the samples into water thin copper wire was used. The density was determined from the following equation:

$$\text{Density} = (M_a / (M_a - M_w)) * 0.988$$

M_a Mass in air and M_w is the Mass in water.

The measurements were carried out in triplicate.

Weight loss measurement

The solubility of bioactive glasses was found by measuring weight loss in SBF at 37.5 $^\circ$ C in the incubator. With the help of 500 grit polishing papers the samples were polished. Then they were washed in acetone for a minute and were placed in small plastic containers which contain SBF. On various days, the samples were taken out and with the help of tissue excess moisture was removed. Then the samples are weighted. The percentage changes in weight loss were directly correlated to glass corrosion or solubility in SBF. The amount of weight loss was calculated using the following equation:

$$\text{Weight loss (mg/g)} = (W_i - W_j) / W_i$$

Where W_i is the initial weight of the specimen and W_j the weight on different days

RESULT AND DISCUSSION

FTIR Absorbance Spectrometric Investigation of bioactive glass

Figure.1 shows the FT-IR absorbance spectra of glass samples before put into SBF solution. From Fig.1 it was trend that G-1 and G-2 are showing almost same peak i.e. at wave number 1338.54384 cm $^{-1}$ respectively. Another peak at 1031 and 945 cm $^{-1}$ are shown by G-1 and G-2. From the infrared absorption band table we can see that these peaks are referring to -O-Si- bond and -Si-P-bond¹⁵. The rest three glasses are showing peaks in the range 1562, 1556 and 1558, 1338 and 945, 943, 947 cm $^{-1}$. The 1338 cm $^{-1}$ wave number corresponds to P=O bond³²⁻³⁸. The wave number 1556 cm $^{-1}$ corresponds to -Si-P- bond³⁹. The FTIR spectra peaks of G-2, G-3, G-4 and G-5 samples

have the same behaviour of G-1 with small change in the peaks intensities as shown in the Fig.1. The glasses doped with Zirconia are not showing remarkable changes in the FTIR absorption spectral characteristics.

In vitro bioactivity by FTIR Absorbance Spectrometric

The FT-IR absorbance spectral bands of the glasses before and after immersion in SBF for different days such as 2, 5, 7, 15, and 31 days were shown in Fig. 2, 6. The increased in the intensity of absorption band is due to increase in molecular concentration of species on the surface of the glasses after soaking into SBF with time^{40, 41}.

The FTIR analysis of G-1 before and after drenched into SBF solution for 2, 5, 7, 15 and 31 days were presented in Fig. 2. The new bands were establish to come out after soaking into 2 days in SBF solution and the corresponding wave numbers are 3745, 1558, 738 cm^{-1} . The FTIR absorbance spectral band at 738 corresponds to Si-O-Si symmetric stretch of tetrahedral non-bridging oxygen atoms¹⁵. The major bands at about 1558 are credited to Si-P bond. On 15th day and 31st day peaks are found out at 3184 cm^{-1} which corresponds to -OH group (associated). The 1296 cm^{-1} wave number corresponds to P=O bond. The band corresponds to wave number 3745 cm^{-1} belongs to hydroxyl group (O-H stretch (free))⁴². This long

duration of the sample soaked into SBF shows small decrease in the intensities of the bands which is the result of formation of hydroxyl apatite (HA) layer⁴³.

Figure. 3 depict it the FT-IR analysis of G-2 both before, after drenched into SBF solution for 2, 5, 7, 15 and 31 days. On 5th days of soaking the prominent wave numbers are obtained at 3136, 1552, 1338, 1028 cm^{-1} . The 1552 corresponds to Si-P bond. Here a new peak was obtained corresponding to 1338 cm^{-1} wave number which is associated with Zr-OH bonding^{37, 38}. This is because bio glass is doped with zirconia. The band corresponding to wave number 1028 cm^{-1} is Si-O-Si stretching bond¹⁵. The bands at 652 cm^{-1} correspond to (carbonate) C-O stretching mode. The wave number 3136 cm^{-1} is 31 days corresponds to OH group (associated). This long duration of the sample soaked into SBF shows small decrease in the intensities of the bands which is the result of formation of hydroxyl carbonate apatite (HA) layer⁴³.

Figure. 4 depicted the FT-IR analysis of G-3 before and after drenched it into SBF for 2, 5, 7, 15 and 31 days. On 5th days of soaking, the prominent wave numbers are obtained at wave number 3745, 3136, 2553, 2158, 1558, 1338, 1016, 652 cm^{-1} respectively. The peak obtained at 1338

Table.1: Composition of base and doped glass

Glass sample \ Composition	SiO ₂ (wt %)	Na ₂ O (wt %)	K ₂ O (wt %)	P ₂ O ₅ (wt %)	MgO (wt %)	CaO (wt %)	ZrO ₂ (wt %)
G-1	53	6	12	4	5	20.0	0.0
G-2	53	6	12	4	5	19.5	0.5
G-3	53	6	12	4	5	19.0	1.0
G-4	53	6	12	4	5	18.5	1.5
G-5	53	6	12	4	5	18.0	2.0

Table. 2: Ion concentration (m M/liter) of SBF

Ions	Na ⁺	K ⁺	Mg ²⁺	Ca ²⁺	HCO ₃ ⁻	HPO ₄ ²⁻	SO ₄ ²⁻	Cl ⁻
SBF	142.0	5.0	1.5	2.5	4.2	1.0	0.5	147.8
WBC	142.0	5.0	1.5	2.5	27.0	1.0	0.5	103.0

is corresponds to Zr-OH bonding^{44,45}. This is because bio glass is doped with zirconia. The band corresponding to wave number 1016 cm^{-1} is Si-O-Si stretching bond¹⁵. The wave number of 1558 cm^{-1} is linked with Si-P bond. The bands at 652 and 2553 cm^{-1} are correspond to (carbonate) C-O stretching mode. The wave number at 3136 and 3745 cm^{-1} are corresponds to OH group (associated) OH group (Free) respectively. This long duration of the sample soaked into SBF shows small decrease in the intensities of the bands which is the result of formation of hydroxyl carbonate apatite (HA) layer⁴³.

Figure. 5 depicted the FTIR analysis of G-4 before and after drenched it into SBF solution for 2, 5, 7, 15 and 31 days. On 15th days of soaking, the prominent wave numbers obtained are at 3348 , 1629 , 1558 , 1398 , 1037 , 628 cm^{-1} . The band corresponding to wave number 1037 is Si-O-Si stretching bond¹⁵. Here the peak obtained at 1398 cm^{-1} wave number is corresponds to Zr-OH bonding^{44,45}. This is because bio glass is doped with zirconia. The wave number of 1558 cm^{-1} is associated with Si-P bond. The bands at 628 cm^{-1} corresponds to (carbonate) C-O stretching mode. The wave number at 3136 cm^{-1} corresponds to OH group (associated). This long duration of the sample soaked into SBF shows small decrease in the intensities of the bands which is the result of formation of hydroxyl carbonate apatite (HA) layer⁴³.

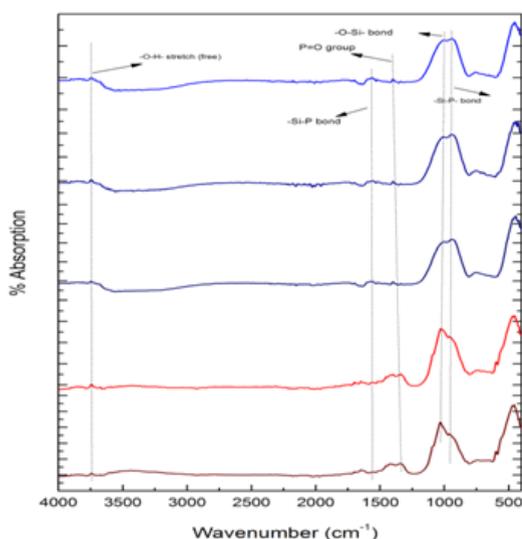


Fig. 1. FTIR Absorbance Spectrometric of glass before immersion into SBF

Figure. 6 depicted the FTIR analysis of G-5 before and after drenched it into SBF solution for 2, 5, 7, 15 and 31 days. The G-3 sample has a peak at wave number 1028 cm^{-1} which corresponds to Si-O-Si symmetric stretching. On 15th day of soaking, the prominent wave numbers obtained are at wave number 3290 , 1629 , 1558 , 1398 , 1037 , 628 cm^{-1} respectively. The band corresponding to wave number 1037 is Si-O-Si stretching bond¹⁵. Here the peak obtained at 1398 cm^{-1} wave number is corresponding to Zr-OH bonding^{44, 45}. This is because bio glass is doped with zirconia. The wave number of 1629 cm^{-1} is associated with Si-P bond. The wave number of 1558 cm^{-1} is associated with P=O bond. The bands at 628 cm^{-1} corresponds to (carbonate) C-O stretching mode. The wave number at 3290 corresponds to OH group (associated). This long duration of the sample soaked into SBF shows small decrease in the intensities of the bands which is the result of formation of hydroxyl carbonate apatite (HA) layer⁴³.

Bioactivity shown by X-ray diffractometer

Figure. 7-11 shows the XRD of the glass samples (G-1, G-2, G-3, G-4 and G-5) before and

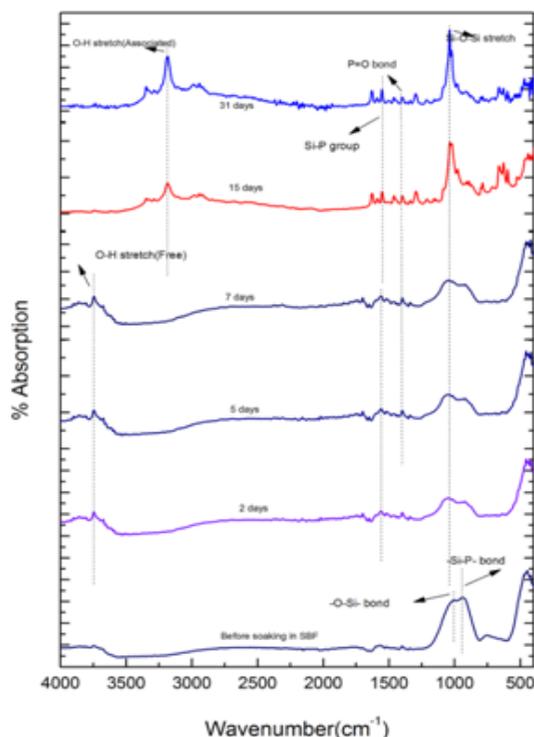


Fig. 2. FTIR of glass sample (G-1) after drenched in SBF

after putting in SBF for 15 days. The bioactive glasses have shown amorphous nature as there is no sharp peak in the XRD before immersion into SBF solution. The graphs which are drawn after soaking into 15 days in SBF solutions showed broad peaks within the range of 30° to 35°.

Figure. 7,11 Contains the XRD pattern of Hydroxy Carbonated Apatite (HCA) powder to compare formation of HA layer on the surface of the SBF treated glass samples⁴⁶. The XRD graphs of the SBF treated samples have clearly shown the formation of crystalline HA on their surfaces after putting in SBF for 15 days. From Fig. 7,11 it was observed with increase in Zirconia content the number of peaks becoming more in numbers and broad. The XRD peak located at around 32° corresponds to Hydroxyapatite $[Ca_{10}(PO_4)_6(OH)_2]$ and the diffraction peaks were matched with the standard PDF#: 740565⁴⁶.

Here it is identified that HA layer is getting formed when zirconia doped 1393 bioactive glasses were immersed into SBF solutions for varying period. The diffraction pattern of all the bioactive glasses showed the Hydroxy Carbonate Apatite crystalline

phases. With increase in zirconia content the peaks which belong to HA were getting sharper.

pH performance in Simulated body fluid

Variation in pH values of bioactive glass after drenched into simulated body fluid (SBF) for 2, 5, 7, 15 and 31 days are presented in Fig. 12. It was observed that up to 7 days all glass samples are showing increase in pH values almost linearly from pH value 7.3. The G-4 bioactive glass is showing maximum pH value on 7th day i.e. 9.5. Due to the addition of zirconia there are variations in the pH values for different samples. The G-4 bioactive glass is showing maximum pH at the end of 31st day. The G-4 which consists of 1.5 wt% of Zirconia is showing maximum pH value. It was found that in all cases the pH value is decreasing after 7 days and attended a constant value up to 31st days. The increase in pH values of bioactive glass in SBF is due to release of Ca^{2+} and Na^+ ions from the sample surface^{22, 23, 25, 26}. The sample number G-4 with higher zirconia content was found to show maximum pH value may be due to high rate of dissolution as compared to base sample G-1. The incorporation of Zirconia into 1393 glass resulted in an increase in the pH of SBF. Their high

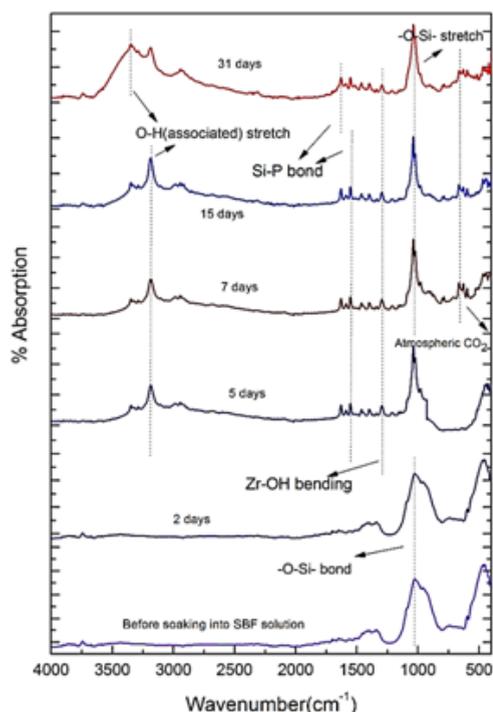


Fig. 3. FTIR of glass sample (G-2) after drenched in Simulated body fluid

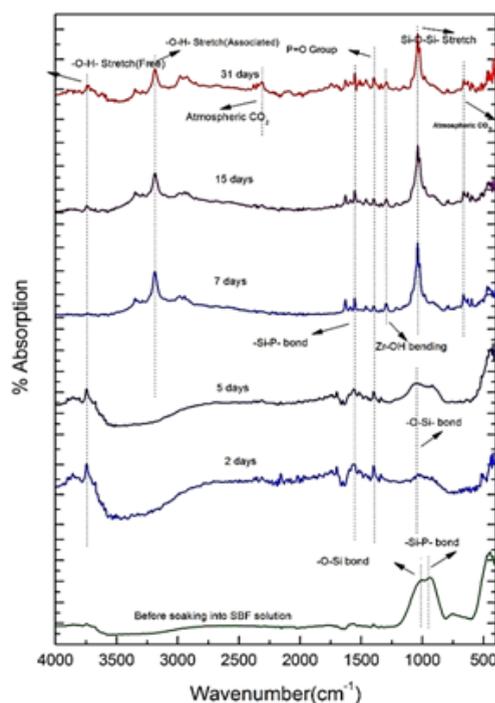


Fig. 4. FTIR of glass sample (G-3) after drenched in Simulated body fluid

degradation rate leads to a higher pH value and this agrees to the condition of formation of HA like layer on the surface of the samples with more crystallinity.

Weight loss measurement

Figure. 13 shows the % weight losses of glasses are presented in Fig. 13. It was observed that the base glass which is un-doped is showing less weight loss after soaking into SBF solutions. In all cases up to 7 days the weight loss is increasing

proportionately with time and after that weight loss is almost constant for respective samples. From the pH graph the inference is already drawn that the pH is increasing due to release of Ca^{2+} and Na^+ ion into SBF solution that means weight loss in G-4 glass is more which is exactly shown by the weight loss graph. It was found from that up to 7 days the weight loss in G-3, G-4 and G-5 glasses are almost same. That means the glasses with higher content of zirconia are possessing higher rate of dissolution, so % weight loss is more.

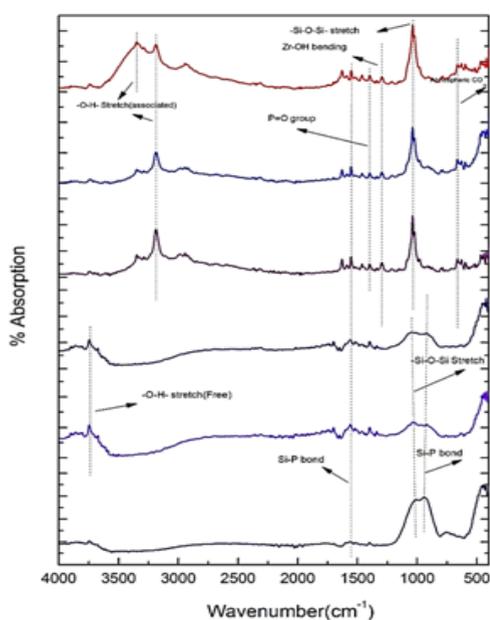


Fig. 5. FTIR of glass sample (G-4) after drenched in Simulated body fluid

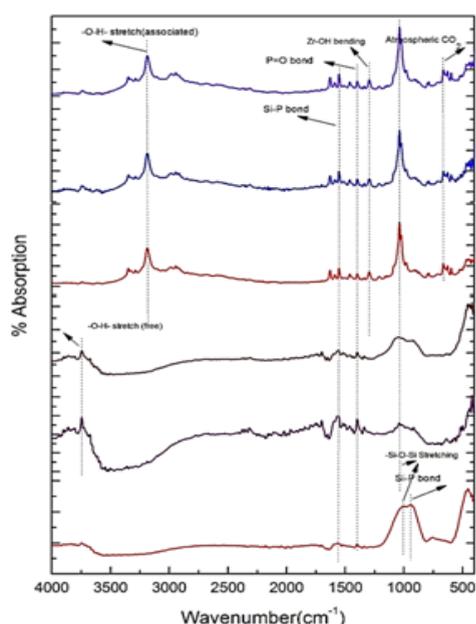


Fig. 6. FTIR of glass sample (G-5) after drenched in Simulated body fluid

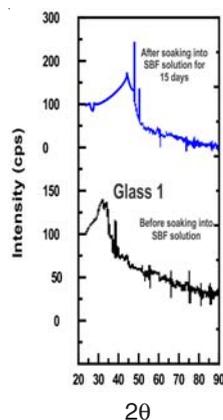


Fig.7

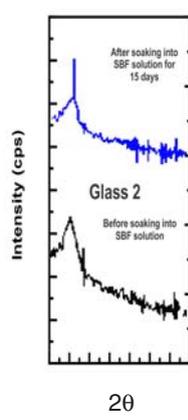


Fig.8

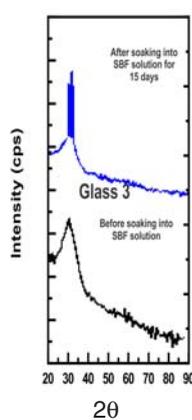


Fig.9

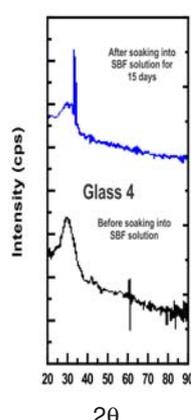


Fig.10

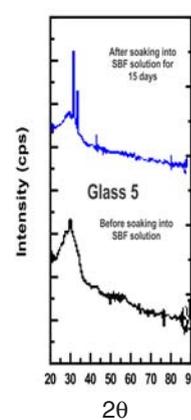


Fig.11

Fig. 7-11. XRD of G-1, G-2, G-3, G-4 and G-5 before and after Simulated body fluid for 15 days

Density Measurement

Figure. 14 shows the density of ZrO_2 doped 1393 glass. It is observed that the density of un-doped sample is found to be 2.44 gm/cm^3 and gradually it is increasing up to G-5 which is the highest doped glass ceramic (2.0 wt% Zirconia). The increase in density is due to replacement of CaO with ZrO_2 which is attributed due to the replacement of a light element (density of CaO – 3.35 g/cm^3) with a heavier one (ZrO_2 – 5.68 g/cm^3). The rate of increment of density is not same in all the cases as the density of G-3 is increased slightly whereas the rate of increment of density from G-3 to G-4 is more. The total range of density is found to be from 2.44 g/cm^3 to 2.495 g/cm^3 . The density of bioactive glass is directly proportional to the solidity of the configuration^{22, 23, 25, 26}. Here G-4 and G-5 glass are showing approximately same density which means these materials are suitable for making scaffolds for bone tissue engineering⁴⁷.

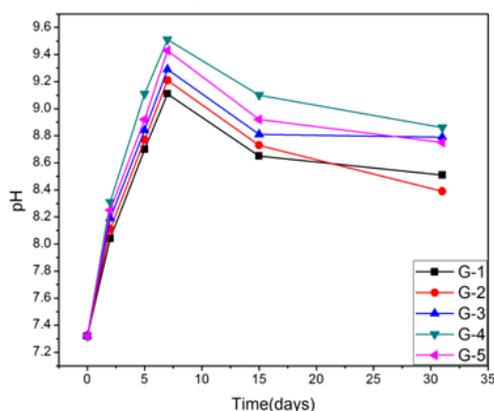


Fig. 12. pH behaviour of 1393 glass and ZrO_2 doped glass sample

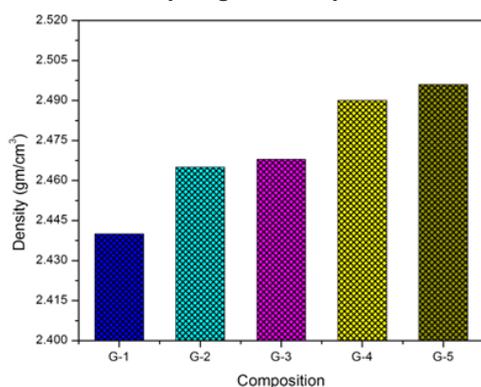


Fig. 14. Density of 1393 bioactive glasses and ZrO_2 doped bioactive glass

Surface morphology by SEM

Figure. 15 and Fig. 16 show the surface morphology of base glass (G-1) and the bioactive glass samples (G-2, G-3, G-4 and G-5) by their SEM images before and after the put in the SBF for 15 days at 37.5°C . The reflections in the SEM images are showing the glassy surfaces. The SEM images of samples before and after put in SBF shown uniform polycrystalline particle on the surfaces of the glass. These developed crystals on the surface of the glasses are assumed to be HCA⁴⁸. It is observed that for increasing ZrO_2 content, the crystalline phase formed is getting uniform shapes on the glass surfaces and for G-5 the hydroxyl carbonate apatite formed is having needle like structure. It can be concluded that the zirconia doped 1393 glasses can also generate HA like structure on their surfaces after immersion in to SBF solution. Previously It was already found out from the XRD

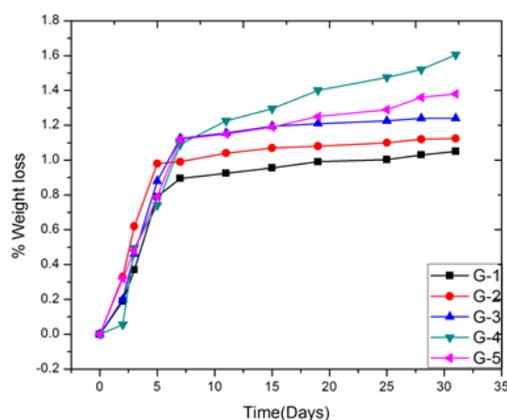


Fig. 13. % Weight loss of 1393 bioactive glass and ZrO_2 doped bioactive glass sample

results and FTIR spectrometry that, the HA layer is getting formed after immersion of glass into SBF for 15 days.

CONCLUSION

The XRD analysis showed the amorphous nature of the glass and FTIR absorbance spectra, pH behaviour; XRD and SEM images show the formation of HCA layer on the surface bioactive glasses after putting in SBF. Densities of substituted bioactive glasses are increased with increasing concentration of ZrO_2 while their Chemical durability decreased.

So it can be concluded from the experimental work that all the ZrO_2 substituted bioactive glass have shown improved properties. Among all the samples the G-4 glass is the best one as it has shown high pH value which suggests

formation of HCA layer. It can be observed from the FTIR diagram of G-4 that all the bonds are showing prominent peaks and SEM images are also showing impressive results of HA layer formation. The prepared bioactive glasses can be used as for bone tissue engineering applications.

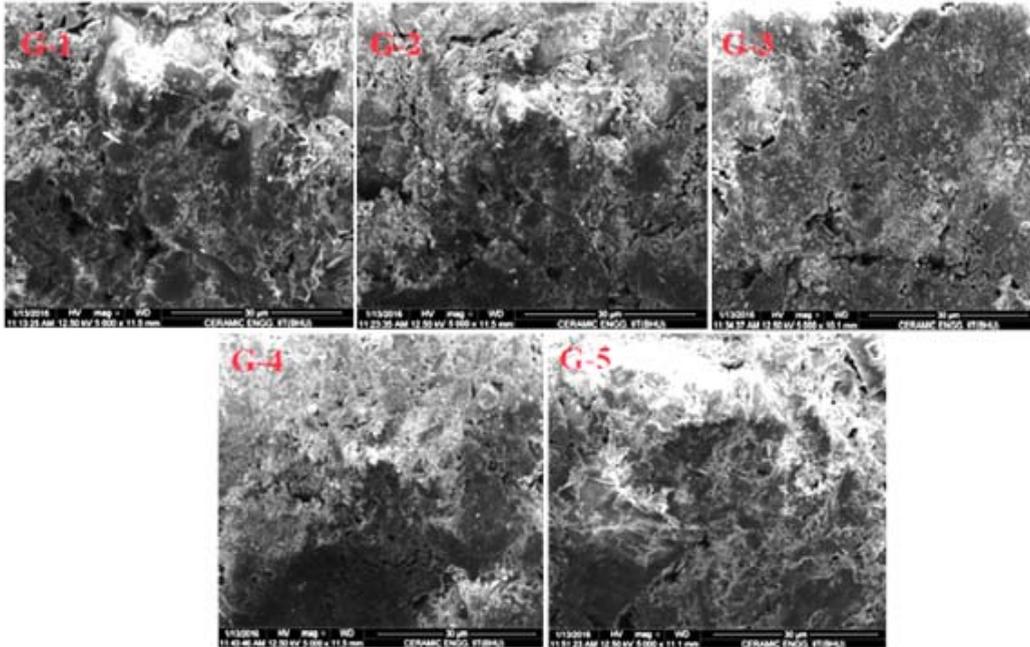


Fig. 15. SEM images of glass before putting into SBF solution

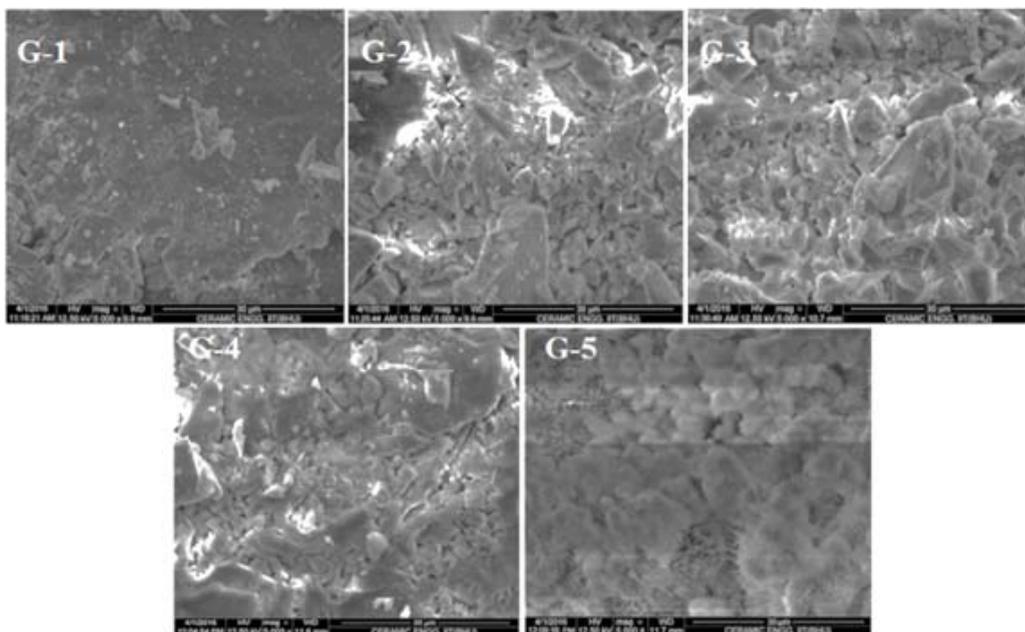


Fig. 16. SEM images of glass after putting into SBF solution

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