

## **CHAPTER-6**

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**Development of zirconia  
substituted 1393 bioactive glass for  
orthopaedic application**

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## 6.1 Introduction

Ceramics which are used for filling and rebuilding of non-healing bone defects and for the healing of damaged or diseased parts of the muscular-skeletal system is termed as bioceramics [Sergey, V. D et al., 2010]. Bio inert (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 composites [Hench, L.L et al. 1998]. Several types of bioactive glasses are developed over the past years. Bioactive glasses (BG) have been known for their bioactive properties and their aptitude to form a strong bond to the bone by the formation of hydroxyapatite surface layer [Hoppe. A.; Jokic B.; Janackovic D, 2014]. Bioactive glasses (BGs) such as “45S5 Bio-glass” ( $45\text{SiO}_2\text{-}24.5\text{CaO-}24.5\text{Na}_2\text{O-}6\text{P}_2\text{O}_5$  wt %) and “1393 Bio-glass” ( $53\text{SiO}_2\text{-}6\text{Na}_2\text{O-}12\text{K}_2\text{O-}5\text{MgO-}20\text{CaO-}4\text{P}_2\text{O}_5$  wt %) compositions have been widely used for bone tissue engineering applications [Hench, L.L et al. 1998, Hoppe. A.; Jokic B.; Janackovic D,2014]. Hench et al. examined the in vitro bonding mechanism with synthetic material because of the chemical reactions taking place over the 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 bone [Ylanen, H. O. et al. 2011]. 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 past [Heness, G.; Ben-Nissan, B. 2004, Watts, S. J.; O'Donnell, M. D.; Law, R. V.; Hill, R. G 2010, Brauer, D. S.; Karpukhina, N.; O'Donnell M. D.; Law, R. V.; Hill, R. G.,2010 Fredholm,Y. C.; Karpukhina, N.; Law, R. V.; Hill, R. G., 2010]. Several types of research 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 mineralization [Fatma, H.; ElBatal; Amany ;ElKheshen,2008, O'Donnell, M. D.; Candarlioglu, P. L.; Miller C. A.; Gentleman, E.; Stevens, M. M. 2010, Balamurugan, A.; Rebelo, A.H.; Lemos, A.F.; Rocha, J.H.; Ventura, J.M.; Ferreira, J.M. 2008, Oki, A.; Parveen, B.; Hossain, S; Adeniji, S.; Donahue, H 2004, Saboori, A.; Sheikhi, M.; Moztarzadeh, F.; Rabiee, M.; Hesaraki, S.; Tahriri, M 2009, Vyas et al. 2014]. The ZrO<sub>2</sub> substituted is widely used as a substrate in hard tissue applications due to its excellent strength and fracture toughness [Hulbert, S.F et al. 1993]. It was discovered from the reaction product obtained after heating gems by the German chemist Martin Heinrich Klaproth in 1789 [Julian Jones, R. et al. 2009]. Several research articles have suggested that zirconia has good chemical and dimensional stability, mechanical strength and toughness and it is also biologically inert [Miao, X.; Hu, Y.; Liu, J.; Huang, X. 2007]. Many studies have shown that the compressive strength of ZrO<sub>2</sub> is relatively higher than porous HA and ZA20 (20 wt. % Al<sub>2</sub>O<sub>3</sub> added TZP) ceramics. In vitro evaluation has also shown that ZrO<sub>2</sub> is not cytotoxic [Sarkar, R et al. 2013, Zhang et al. 2010, Ducheyne 1980]. [Vyas et al. 2015] 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) had increased physicomechanical properties and bioactivity of their samples due to formation Co-O-Si and Ni-O-Si bonds. Tripathi et al. [Tripathi H.; Kumar A. S.; Singh S. P, 2016] have also found the characterization of Li<sub>2</sub>O–CaO–Al<sub>2</sub>O<sub>3</sub>–P<sub>2</sub>O<sub>5</sub>–SiO<sub>2</sub> glasses as bioactive material and all the elastic moduli values were established to increase with the increase in Al<sub>2</sub>O<sub>3</sub>/Li<sub>2</sub>O ratio. In the current investigation, an endeavor has been made to study the effect of zirconia doping in silica-based bioactive glass and to verify its bioactivity and other characteristics.

## 6.2 Material and methods

### 6.2.1 Preparation of bioactive glasses

For the source of SiO<sub>2</sub> [Hoppe et al. 2014], Na<sub>2</sub>O, CaO, K<sub>2</sub>O, P<sub>2</sub>O<sub>5</sub> and MgO, Fine-grained quartz, anhydrous sodium carbonate [Na<sub>2</sub>CO<sub>3</sub>], anhydrous calcium carbonate [CaCO<sub>3</sub>], Potassium Carbonate [K<sub>2</sub>CO<sub>3</sub>], ammonium dihydrogen orthophosphate [NH<sub>4</sub>H<sub>2</sub>PO<sub>4</sub>] and Magnesium Carbonate [MgCO<sub>3</sub>] respectively were used [Hoppe et al. 2014]. The ZrO<sub>2</sub> is available as it is and was added for the 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 an alumina crucible and placed in an electric furnace. The furnace temperature was set to 1400±5°C, and the duration to reach 1400°C was set to 3 hours, and after reaching 1400°C the steady-state was maintained for more 3 hours. After melting the prepared bioactive glass samples were poured into steel molds and were directly transferred to a regulated muffle furnace at the temperature of 470°C for annealing. The muffle furnace was allowed to cool down to room temperature at a controlled rate of 100°C/h. Annealing is done to remove the internal stress after the glasses are formed.

Table 6.1- Composition of base and doped bioactive glasses (wt%)

	SiO <sub>2</sub>	Na <sub>2</sub> O	K <sub>2</sub> O	P <sub>2</sub> O <sub>5</sub>	MgO	CaO	ZrO <sub>2</sub>
<b>1393</b>	53	6	12	4	5	20.0	0.0
<b>G-1</b>	53	6	12	4	5	19.5	0.5
<b>G-2</b>	53	6	12	4	5	19.0	1.0
<b>G-3</b>	53	6	12	4	5	18.5	1.5
<b>G-4</b>	53	6	12	4	5	18.0	2.0

### 6.2.2 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 [Vyas et al. 2015]. The ion concentrations of SBF are given on **Table 6.2**.

#### 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  $N_1, N_2$  = Normality of 1<sup>st</sup> and 2<sup>nd</sup> solution respectively  
 $V_1, V_2$  = Volume of 1<sup>st</sup> and 2<sup>nd</sup> 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 p<sup>H</sup> was adjusted to be within 7.2/7.25.

Thus, SBF solution was prepared which was used forevaluation of bioactivity of artificial materials in vitro.

Table 6.2- Ion concentration (mM/litre) of simulated body fluid and human blood plasma

Ion	Na <sup>+</sup>	K <sup>+</sup>	Mg <sup>2+</sup>	Ca <sup>2+</sup>	HCO <sub>3</sub> <sup>-</sup>	HPO <sub>4</sub> <sup>2-</sup>	SO <sub>4</sub> <sup>2-</sup>	Cl <sup>-</sup>
Simulated body fluid	142.0	5.0	1.5	2.5	4.2	1.0	0.5	147.8
Human blood plasma	140.0	5.0	1.5	2.5	27.0	1.0	0.5	103.0

### 6.2.3 XRD and FTIR analysis

Using the XRD, crystalline phases present in before and after SBF treated glass samples were identified. The samples were ground to 75 $\mu$ m and subjected to XRD using RIGAKU-Miniflex II diffractometer of range between 20° and 80°. 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<sup>-1</sup>. The surface morphology of bioactive glass samples was analyzed by SEM (Inspect S50, FEI) before and after SBF behavior.

### 6.2.4 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°C in a static condition for 2, 5, 7, 15 and 31 days. pH was also measured using Universal Bio microprocessor 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 analyzed by SEM (Inspect S50, FEI) before and after SBF behavior. 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°C. Samples were coated with gold before SEM analysis.

### 6.2.5 Density measurement

The density of samples was measured by the 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.

### 6.2.6 Weight loss measurement

The solubility of bioactive glasses was found by measuring weight loss in SBF at 37.5°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 percentages of 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:

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

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

## 6.3 Result and discussion

### 6.3.1 FTIR absorbance spectrometric investigation of bioactive glasses

**Figure 6.1** shows the FT-IR absorbance spectra of glass samples before putting into SBF solution. From Figure-1, it was observed that 1393 and G-1 are showing almost the same peak i.e., at wave number 1338.54384  $\text{cm}^{-1}$  respectively. G-1 and G-2 show another peak at 1031 and 945  $\text{cm}^{-1}$ . From the infrared absorption band table, we can see that these peaks are referring to -O-Si- bond and -Si-P- bond [Vyas et al. 2014]. The rest three glasses are showing peaks in the range 1562, 1556 and 1558, 1338 and



945, 943, 947  $\text{cm}^{-1}$ . The 1338  $\text{cm}^{-1}$  wave number corresponds to the P=O bond [Montagne et al. 1996]. The wave number 1556  $\text{cm}^{-1}$  corresponds to -Si-P- bond [Abel, E. W.; Sabherwal I. H. 1968]. The FTIR spectra peaks of G-1, G-2, G-3, and G-4 samples have the same behavior as that of 1393 with a small change in the intensities of the peaks, as shown in the **Figure.-6.1**. The glasses doped with Zirconia are not showing remarkable changes in the FTIR absorption spectral characteristics.

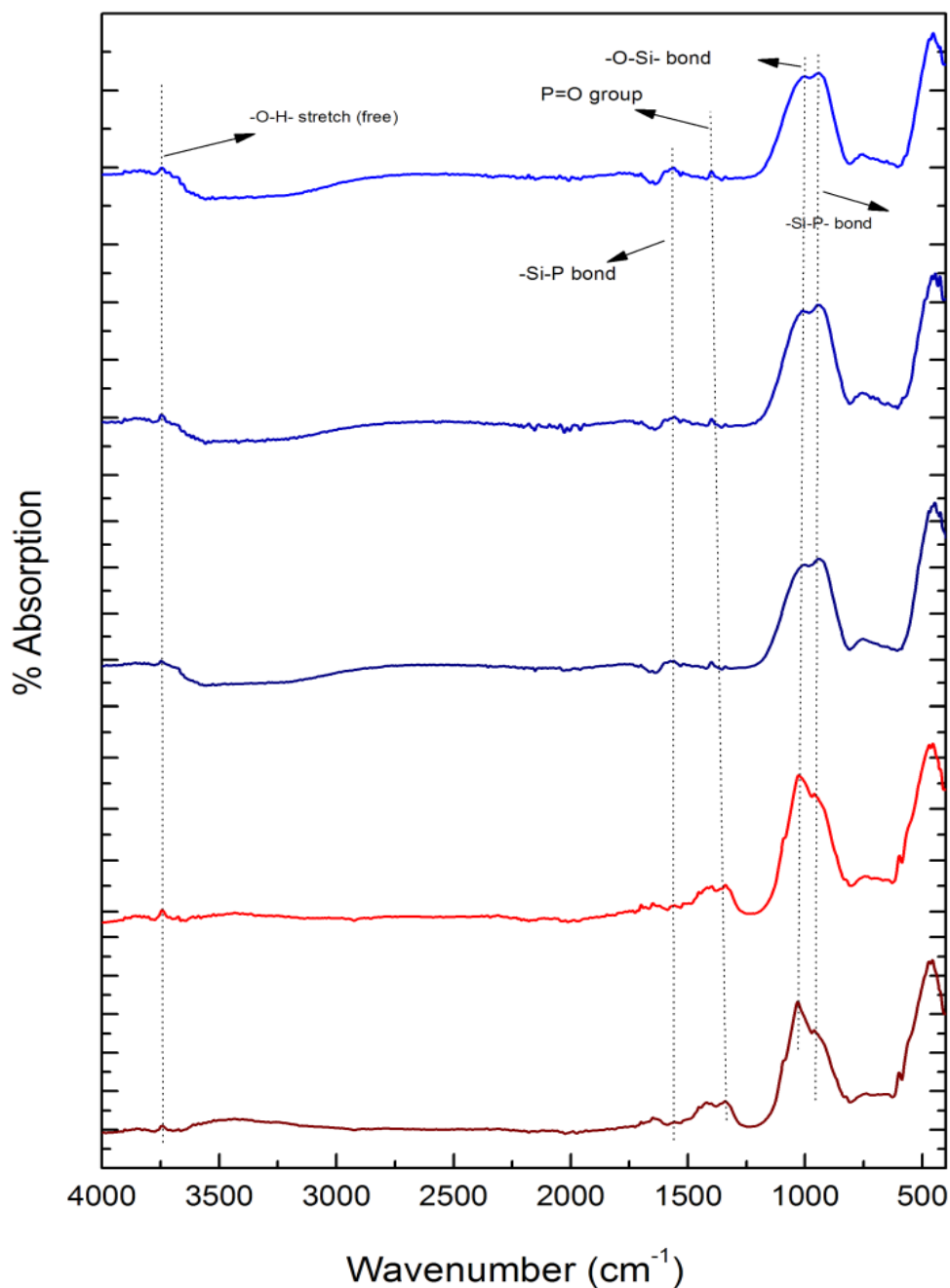


Figure 6.1- FTIR absorbance spectrometry of bioactive glasses before immersion in SBF

### 6.3.2 In vitro bioactivity by FTIR absorbance spectrometry

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 Figure 6.2 to 6.6. The increased in the intensity of the absorption band is due to an increase in the molecular concentration of species on the surface of the glasses after soaking into SBF with time. [Rehman, I.; Karsh, M.; Hench, L.L.; Bonfield, W.; 2000 and Filgueiras, M.R.; LaTorre, G.; Hench, L.L. 1993].

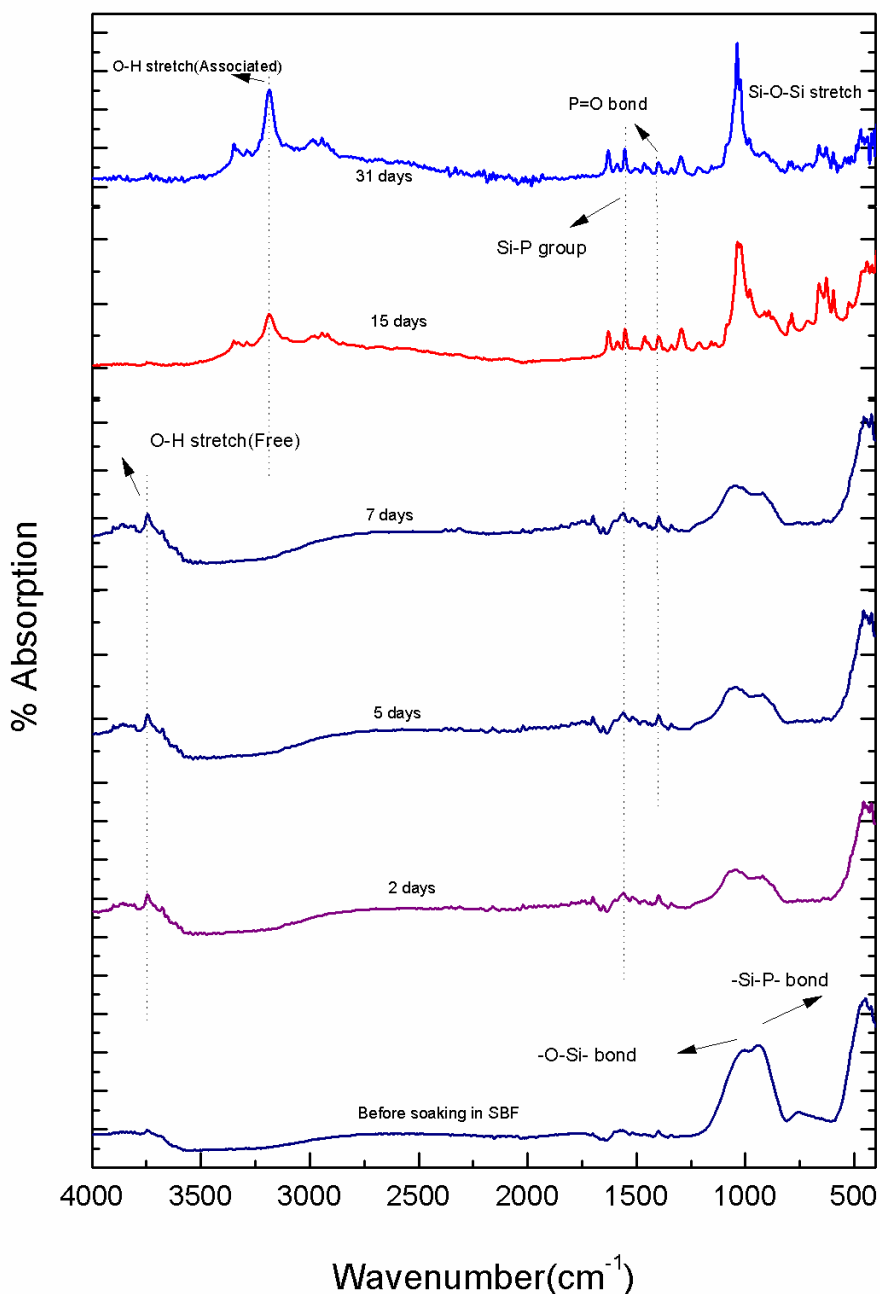


Figure 6.2- FTIR of 1393 bioactive glass after drenched in SBF

The FTIR analysis of 1303 before and after soaked into SBF solution for 2, 5, 7, 15, and 31 days were presented in **Figure-6.2**. The new bands established to come out after soaking into 2 days in SBF solution and the corresponding wave numbers are 3745, 1558, 738  $\text{cm}^{-1}$ . The FTIR absorbance spectral band at 738 corresponds to Si-O-Si symmetric stretch of tetrahedral non-bridging oxygen atoms [Vyas et al. 2014]. The major bands at about 1558 are credited to Si-P bond. On 15th day and 31st-day peaks are found out at 3184  $\text{cm}^{-1}$  which corresponds to -OH group (associated). The 1296  $\text{cm}^{-1}$  wave number corresponds to the P=O bond. The band corresponds to wave number 3745  $\text{cm}^{-1}$  belongs to a hydroxyl group (O-H stretch (free)) [Nayak, J. P.; Kumar, S.; J. Bera 2010]. 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 hydroxylapatite (HA) layer [Arepalli S. K.; , Tripathi, H.; Vyas, V. K.; Jain, S.; Suman, S. K.; Ram Pyare; Singh, S.P, 2015].

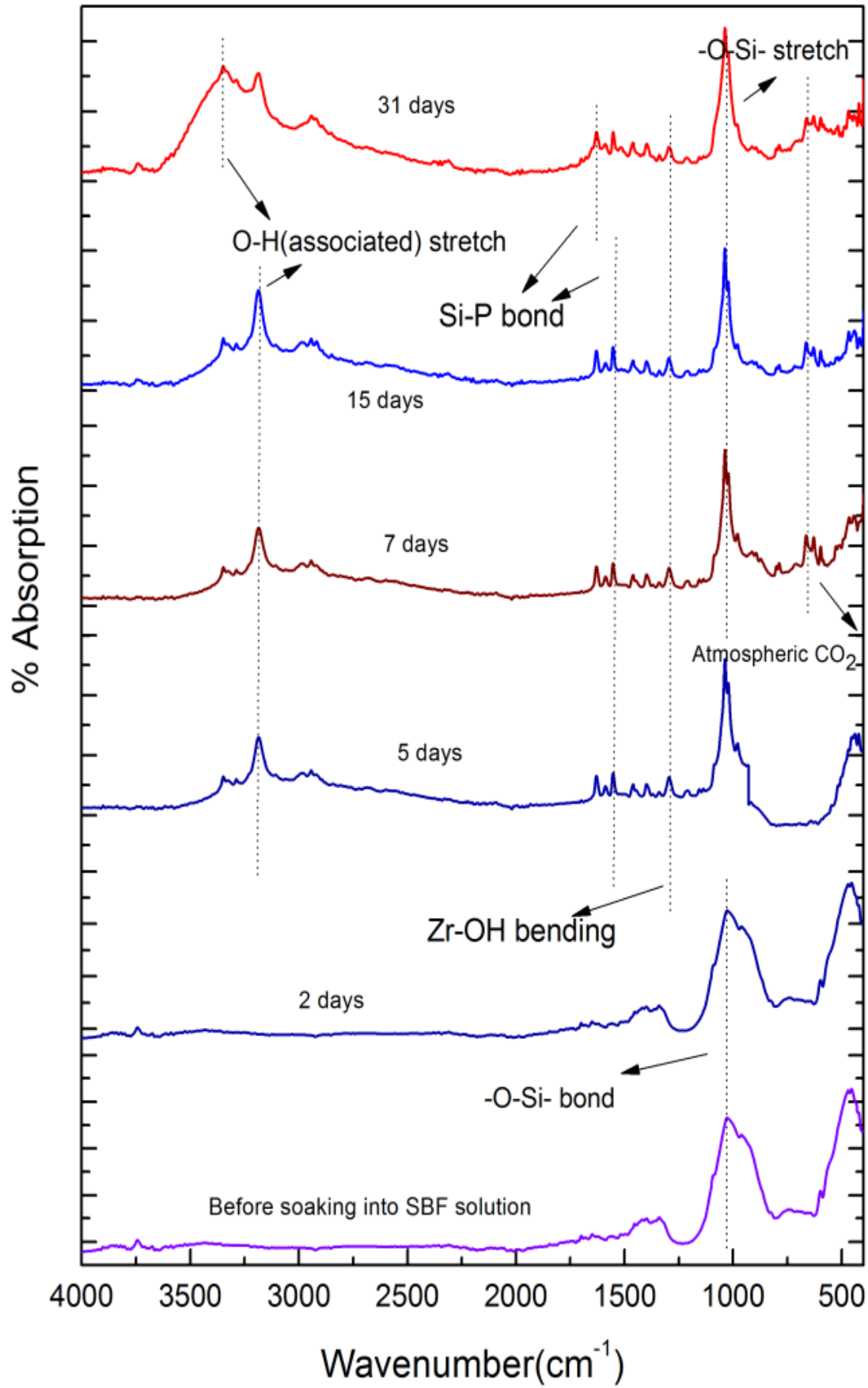


Figure 6.3- FTIR of bioactive glass sample (G-1) after drenched in SBF

**Figure.6.3** depicts the FT-IR analysis of G-1 both before, after soaking into the 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  $\text{cm}^{-1}$ . 1552 wavenumber corresponds to Si-P bond. Here a new peak was obtained corresponding to 1338  $\text{cm}^{-1}$  wavenumber, which is associated with Zr-OH bonding [Chahine, A et al.2004]. This is because bioglass is doped with zirconia. The band corresponding to wave number 1028  $\text{cm}^{-1}$  is Si-O-Si stretching bond [Arepalli S. K.; , Tripathi, H.; Vyas, V. K.; Jain, S.; Suman, S. K.; Ram Pyare; Singh, S.P, 2015]. The bands at 652  $\text{cm}^{-1}$  correspond to (carbonate) C-O stretching mode. The wavenumber 3136  $\text{cm}^{-1}$  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) layer [Arepalli S. K.; , Tripathi, H.; Vyas, V. K.; Jain, S.; Suman, S. K.; Ram Pyare; Singh, S.P, 2015].

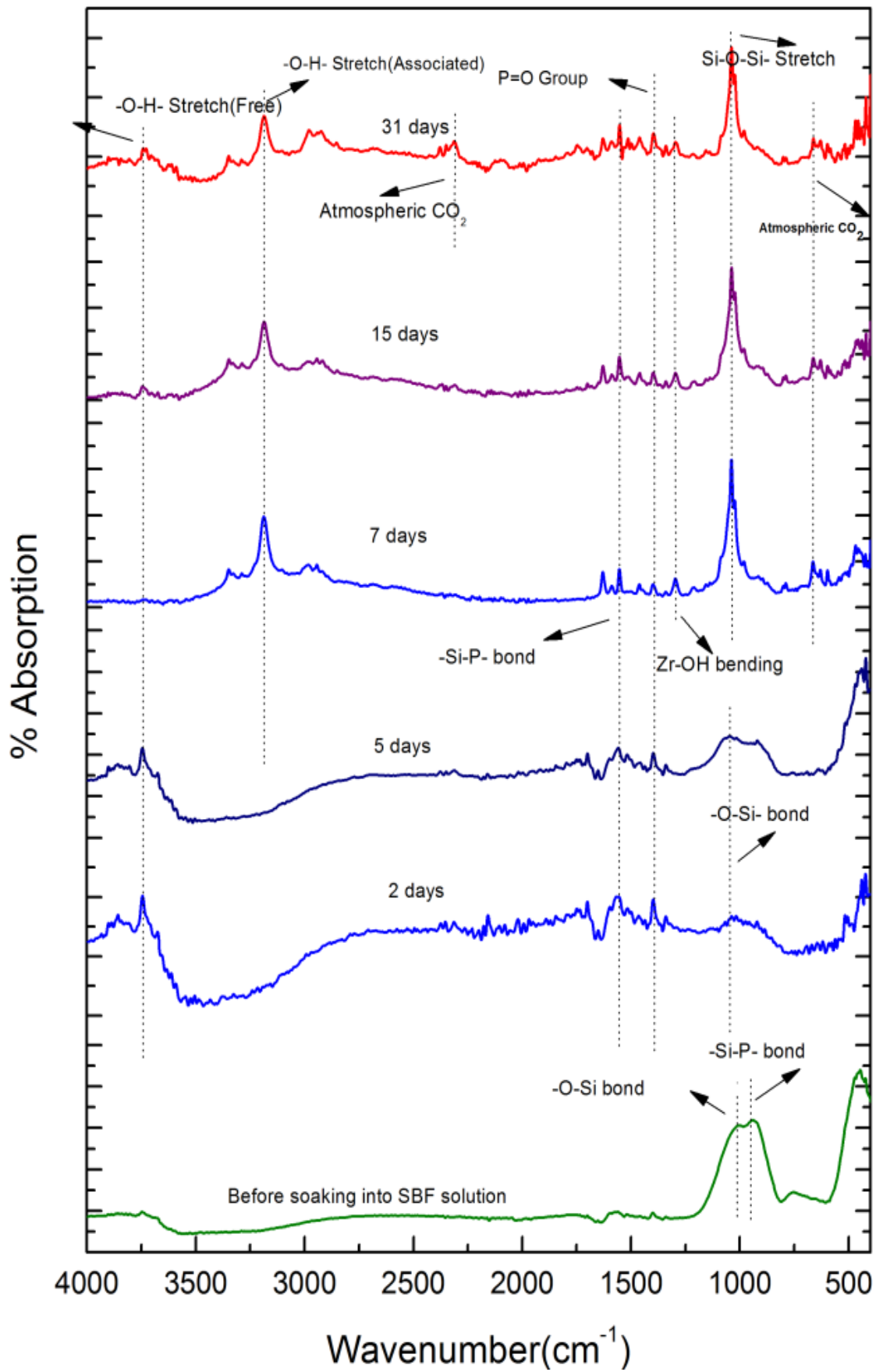


Figure 6.4- FTIR of bioactive glass sample (G-2) after drenched in SBF

**Figure.6.4** depicted the FT-IR analysis of G-2 before and after soaked 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  $\text{cm}^{-1}$  respectively. The peak obtained at 1338 correspond to Zr-OH bonding [Jayakumar, S.; 2011 and Sarkar, D 2007]. This is because bioglass is doped with zirconia. The band corresponding to wave number 1016  $\text{cm}^{-1}$  is Si-O-Si stretching bond [Vyas et al. 2014]. The wavenumber of 1558  $\text{cm}^{-1}$  is linked with Si-P bond. The bands at 652 and 2553  $\text{cm}^{-1}$  are correspond to (carbonate) C-O stretching mode. The wave number at 3136 and 3745  $\text{cm}^{-1}$  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) layer [Arepalli S. K.; , Tripathi, H.; Vyas, V. K.; Jain, S.; Suman, S. K.; Ram Pyare; Singh, S.P, 2015].

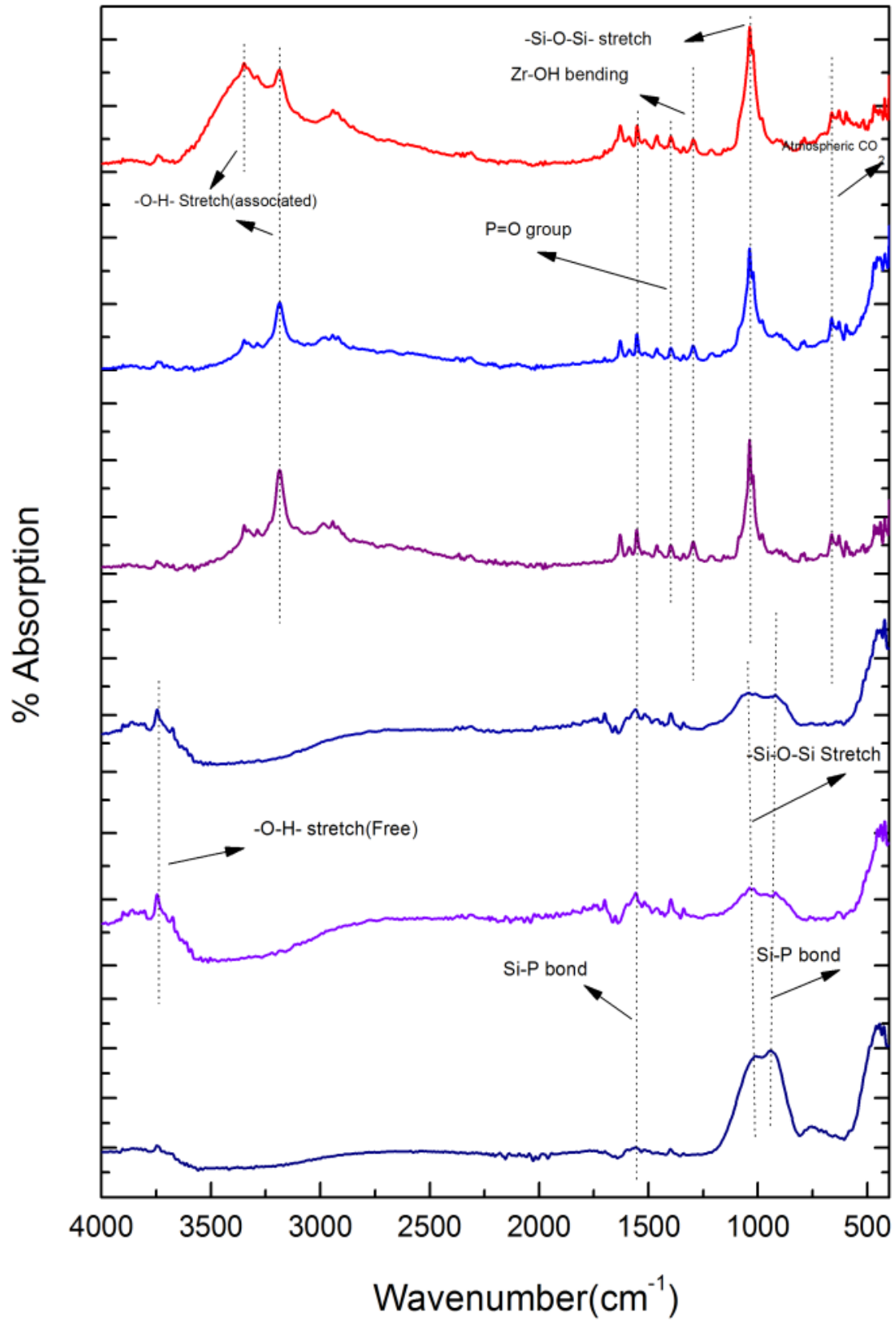


Figure 6.5- FTIR of bioactive glass sample (G-3) after drenched in SBF



**Figure 6.5** depicted the FTIR analysis of G-3 before and after soaked 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  $\text{cm}^{-1}$ . The band corresponding to wave number 1037  $\text{cm}^{-1}$  is the Si-O-Si stretching. Here the peak obtained at 1398  $\text{cm}^{-1}$  wave number is corresponds to Zr-OH bonding [Jayakumar, S.; Ananthapadmanabhan, P.V.; Perumal, K.; Thiyagarajan, T.K.; Mishra, S.C.; Su L.T.; Tok, A.I.Y.; J Guo, 2011 and Sarkar, D.; Mohapatra, D.; Ray, S.; Bhattacharyya S.; Adak, S.; Mitra, N.,2007].

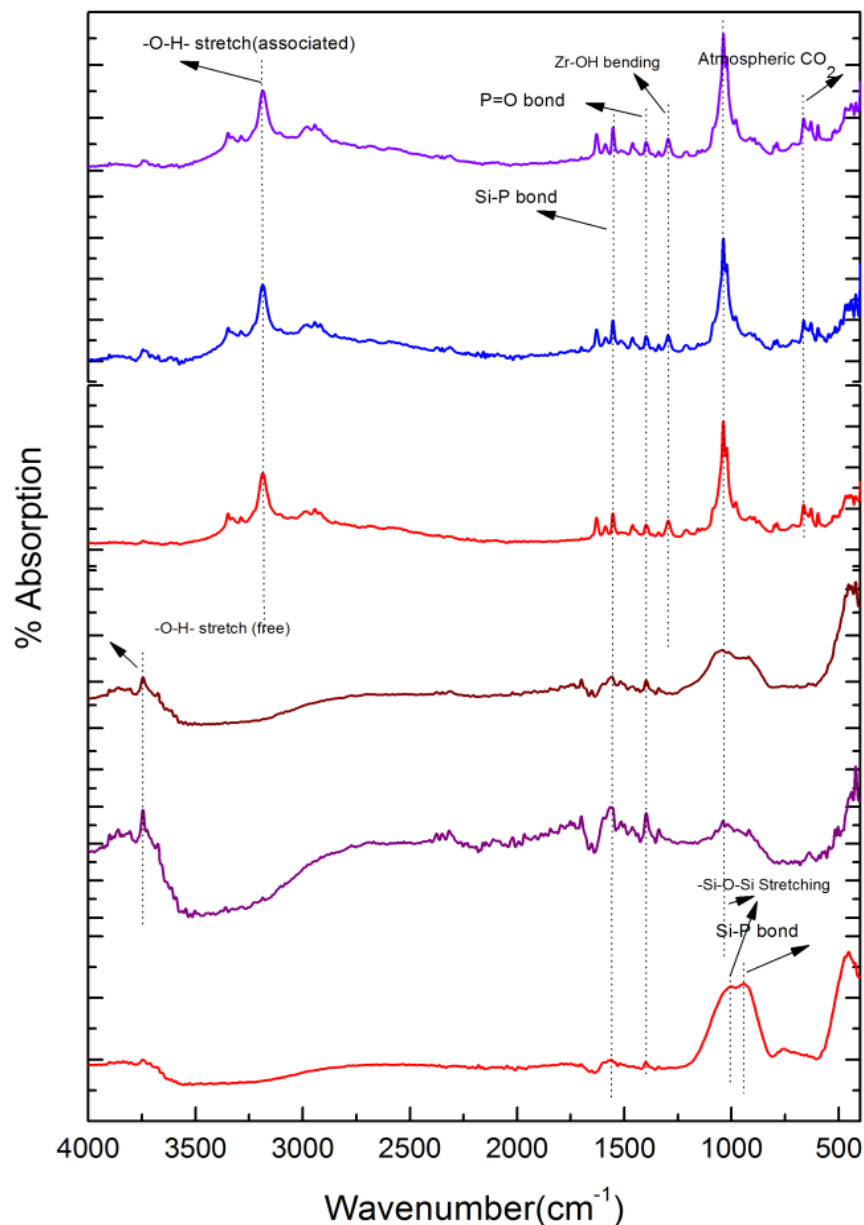
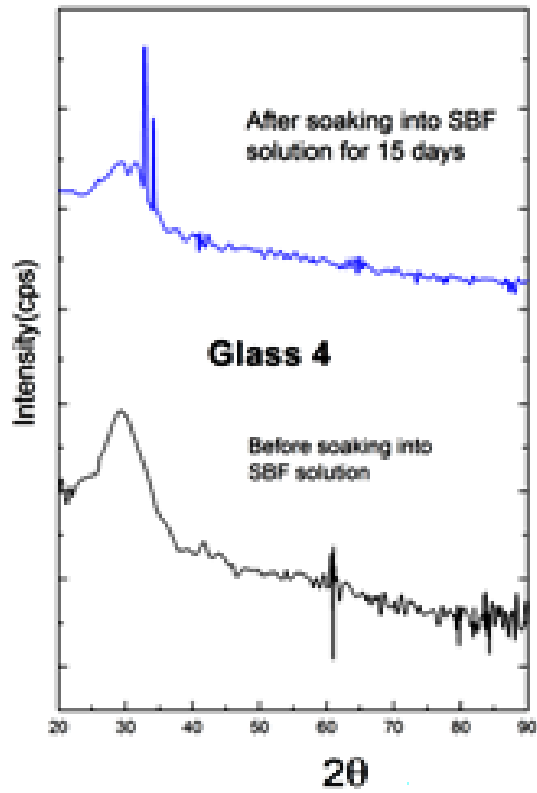
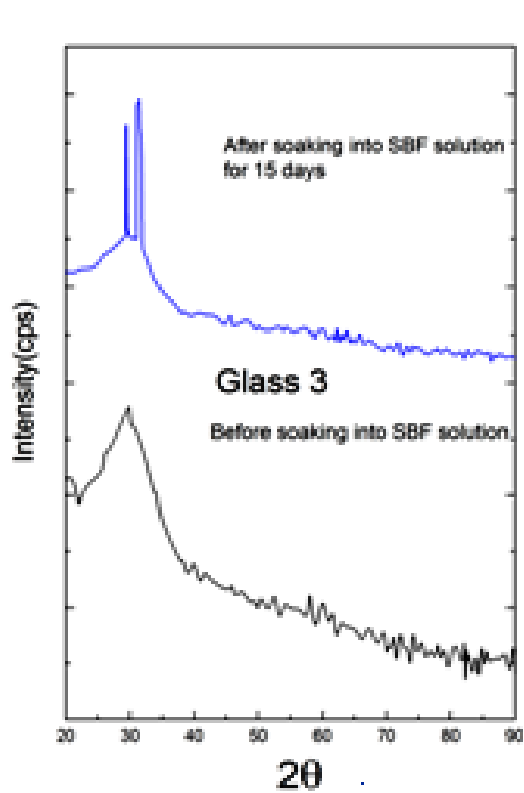
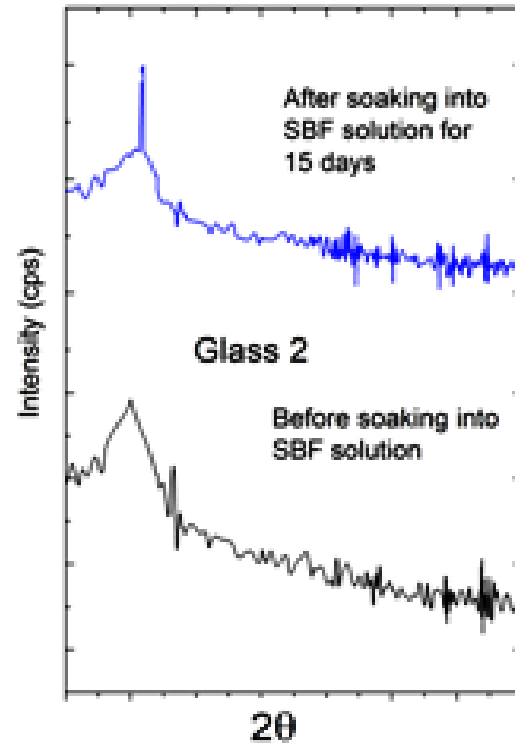
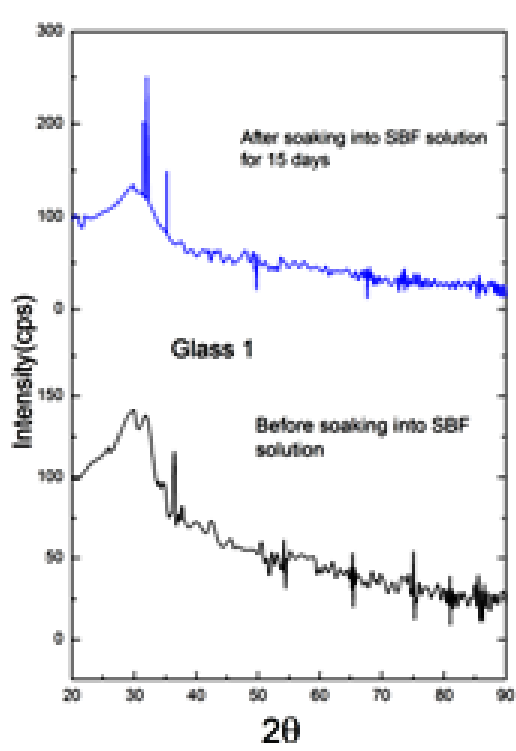


Figure 6.6- FTIR of bioactive glass sample (G-4) after drenched in SBF

**Figure 6.6** depicted the FTIR analysis of G-4 before and after soaked it into SBF solution for 2, 5, 7, 15, and 31 days. The G-4 sample has a peak at wave number  $1028\text{ cm}^{-1}$  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,  $628\text{ cm}^{-1}$  respectively. The band corresponding to wave number  $1037\text{ cm}^{-1}$  is the Si-O-Si stretching bond [Vyas et al. 2014]. Here the peak obtained at  $1398\text{ cm}^{-1}$  wave number is corresponding to Zr-OH bonding [Jayakumar, S.; Ananthapadmanabhan, P.V.; Perumal, K.; Thiyagarajan, T.K.; Mishra, S.C.; Su L.T.; Tok, A.I.Y.; J Guo, 2011 and Sarkar, D.; Mohapatra, D.; Ray, S.; Bhattacharyya S.; Adak, S.; Mitra, N.,2007]. This is because bioglass is doped with zirconia. The wave number of  $1629\text{ cm}^{-1}$  is associated with Si-P bond. The wave number of  $1558\text{ cm}^{-1}$  is associated with P=O bond. This is because bioglass is doped with zirconia. The wave number of  $1558\text{ cm}^{-1}$  is associated with Si-P bond. The bands at  $628\text{ cm}^{-1}$  correspond to (carbonate) C-O stretching mode. The wave number at  $3136\text{ cm}^{-1}$  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) layer [Arepalli S. K.; , Tripathi, H.; Vyas, V. K.; Jain, S.; Suman, S. K.; Ram Pyare; Singh, S.P.; 2015].

### 6.3.3 Bioactivity shown by X-ray diffractometer

Figure 6.7 to 6.11 shows the XRD of the glass samples (1393, G-1, G-2, G-3 and G-4) before and 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  $30^{\circ}$  to  $35^{\circ}$ .



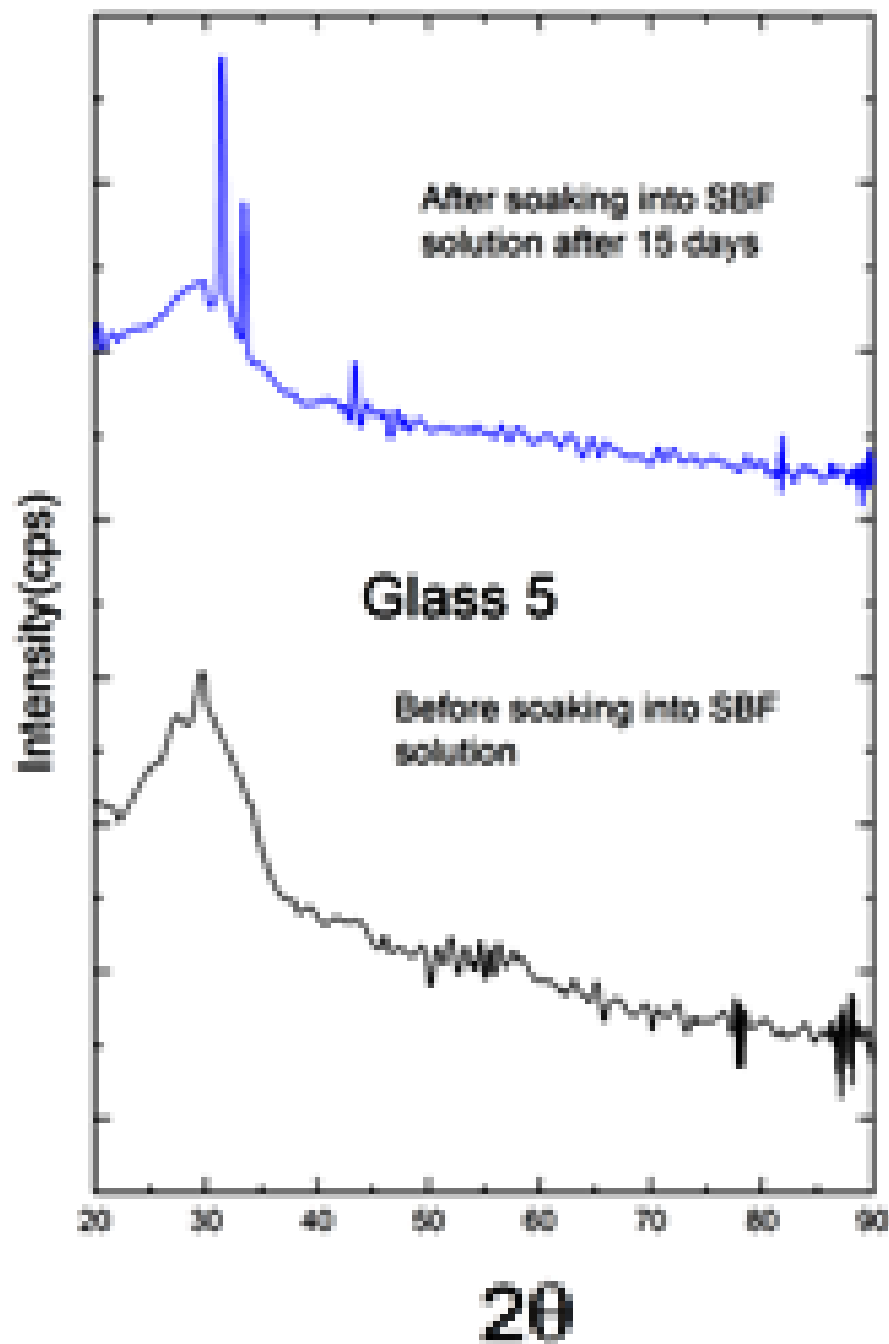


Figure 6.7- XRD of 1393, G-1, G-2, G-3 and G-4 bioactive glasses before and after immersion in SBF for 15 days

**Figure.6.7** Contains the XRD pattern of Hydroxyl Carbonate Apatite (HCA) powder to compare the formation of HA layer on the surface of the SBF treated glass samples [JCPDS Card no. 1994]. 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 **Figure 6.7**, it was observed with increase in Zirconia content the number of peaks becoming more in numbers and broad. The XRD peak located at around  $32^\circ$  corresponds to hydroxyapatite  $[\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2]$  and the diffraction peaks were matched with the standard PDF#: 740565 [JCPDS Card no. 1994].

Here it is identified that the HA layer is formed when zirconia doped 1393 bioactive glasses were immersed into SBF solutions for the varying period. The diffraction pattern of all the bioactive glasses showed the Hydroxyl Carbonate Apatite crystalline phases. With an increase in zirconia content, the peaks which belong to HA have become sharper.

### 6.3.4 pH performance in SBF

Variation in pH values of bioactive glass after soaked into simulated body fluid (SBF) for 2, 5, 7, 15, and 31 days are presented in **Figure 6.8**. It was observed that up to 7 days, all-glass samples are showing an increase in pH values almost linearly from pH value 7.4. The G-3 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-3 bioactive glass is showing maximum pH at the end of 31st day. The G-3, 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  $\text{Ca}^{2+}$  and  $\text{Na}^+$  ions from the sample surface [Vyas et al. 2016]. The sample number G-3 with higher zirconia content was found to show maximum pH value may be due to the high rate of dissolution as compared to base sample 1393. The

incorporation of Zirconia into 1393 glass resulted in an increase in the pH of SBF. Their high degradation rate leads to higher pH value, and this agrees to the condition of formation of HA like layer on the surface of the samples with more crystallinity.

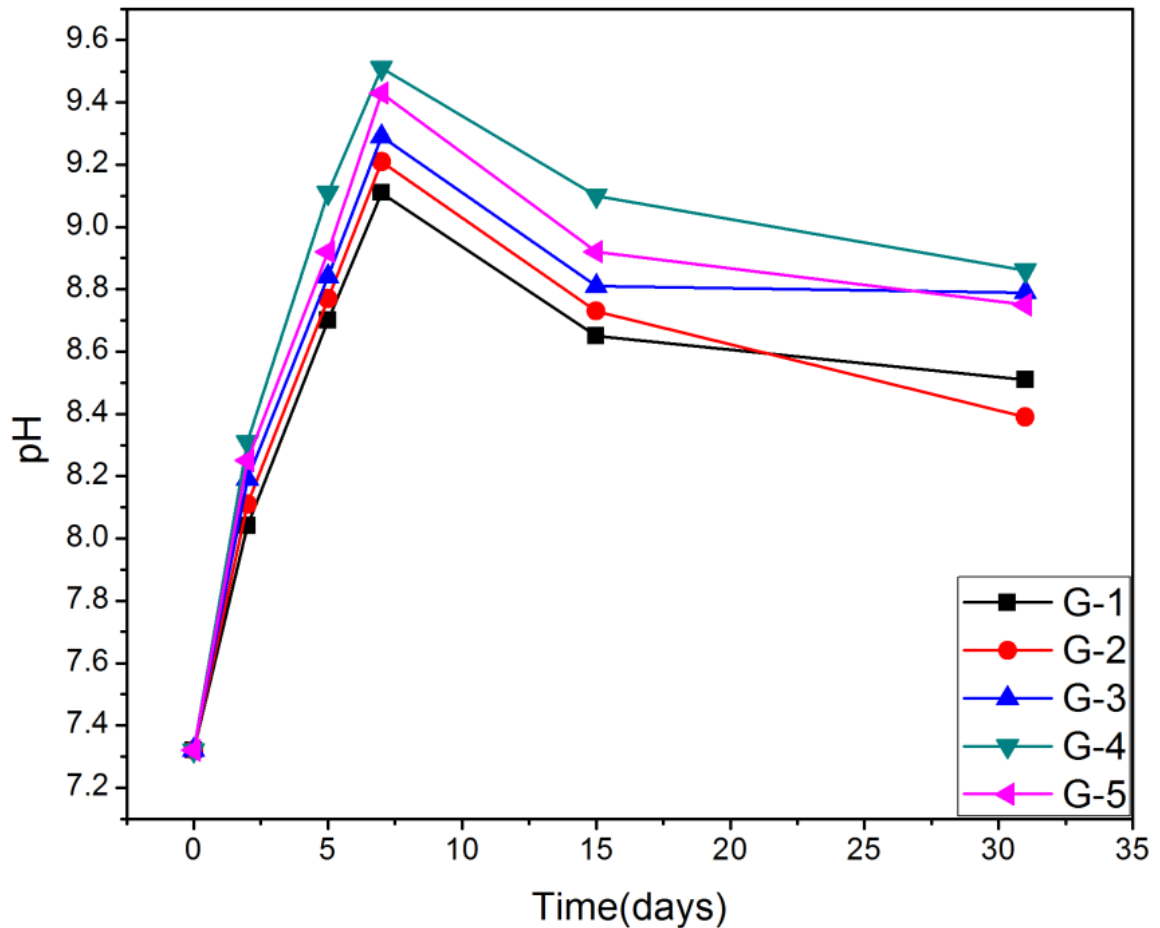


Figure 6.8- pH behavior of 1393 glass and ZrO<sub>2</sub> doped bioactive glasses

### 6.3.5 Weight loss measurement

Figure 6.9 shows the % weight losses of glasses are presented in Figure 6.9. 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  $\text{Ca}^{2+}$  and  $\text{Na}^+$  ion into SBF solution that means weight loss in G-3 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-2, G-3 and G-4 glasses are almost the same. That is why glasses with a higher content of zirconia possess a higher rate of dissolution, so % weight loss is more.

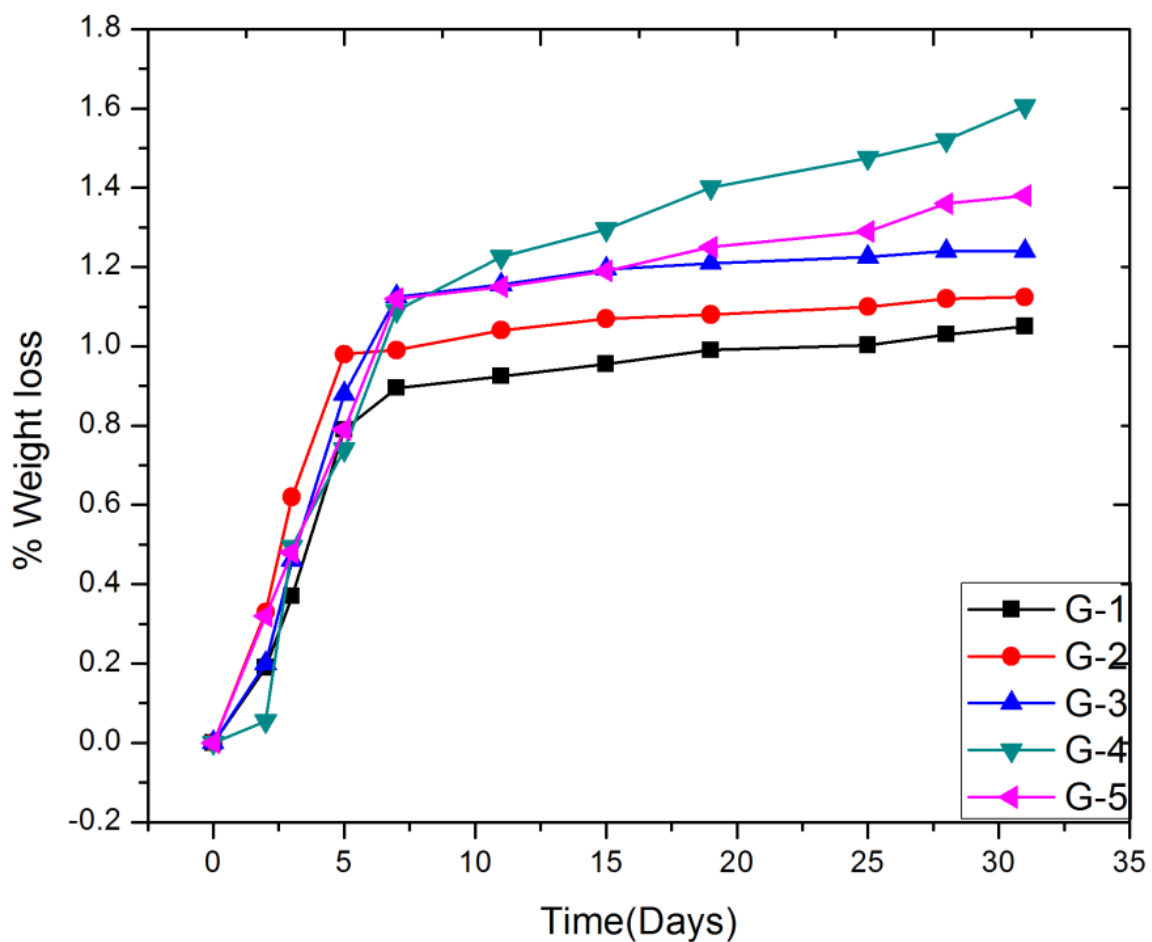


Figure 6.9- % weight loss of 1393 bioactive glass and  $\text{ZrO}_2$  doped bioactive glasses



### 6.3.6 Density measurement

**Figure 6.10** shows the density of ZrO<sub>2</sub> doped 1393 glass. It is observed that the density of the un-doped sample is found to be 2.44 gm/cm<sup>3</sup>, and gradually it is increasing up to G-4 which is the highest doped glass-ceramic (2.0 wt% Zirconia). The increase in density is due to the replacement of CaO with ZrO<sub>2</sub> which is attributed due to the replacement of a light element (density of CaO– 3.35 gm/cm<sup>3</sup>) with a heavier one (ZrO<sub>2</sub> – 5.68 gm/cm<sup>3</sup>). The rate of increment of density is not the same in all the cases as the density of G-2 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 gm/cm<sup>3</sup> to 2.495 gm/cm<sup>3</sup>. The density of bioactive glass is directly proportional to the solidity of the configuration [Vyas et al. 2016]. Here G-3 and G-4 glass are showing approximately same density which means these materials are suitable for making scaffolds for bone tissue engineering [Kansal, I.; Goel A.; Tulyaganov, D.U.; Santos, L.F.; Ferreira, J.M.F, 2011].

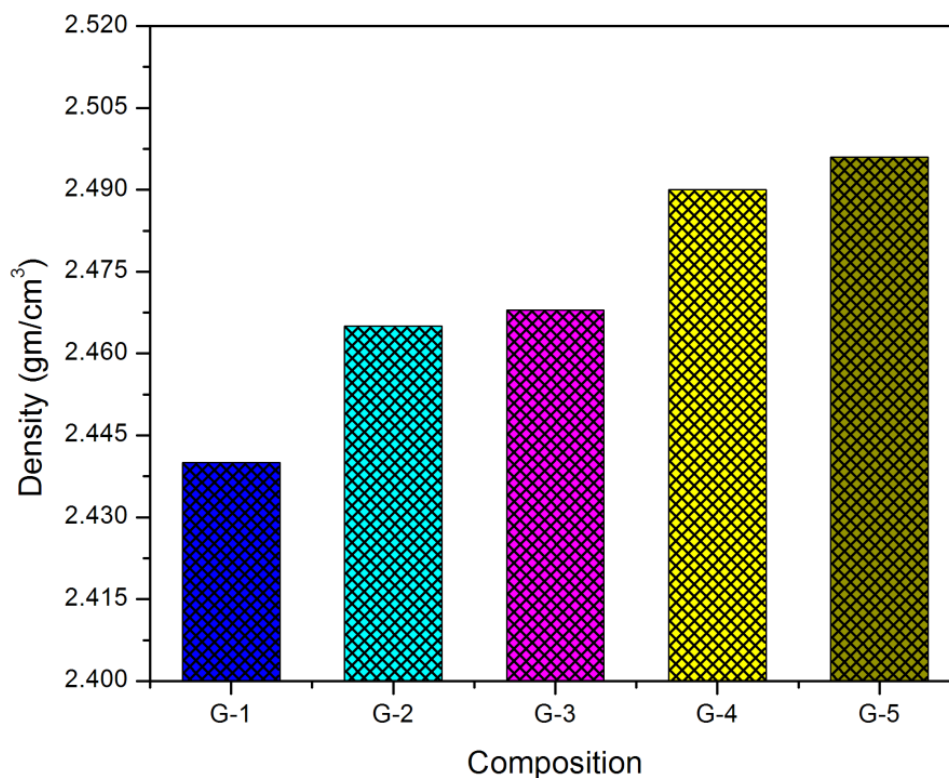
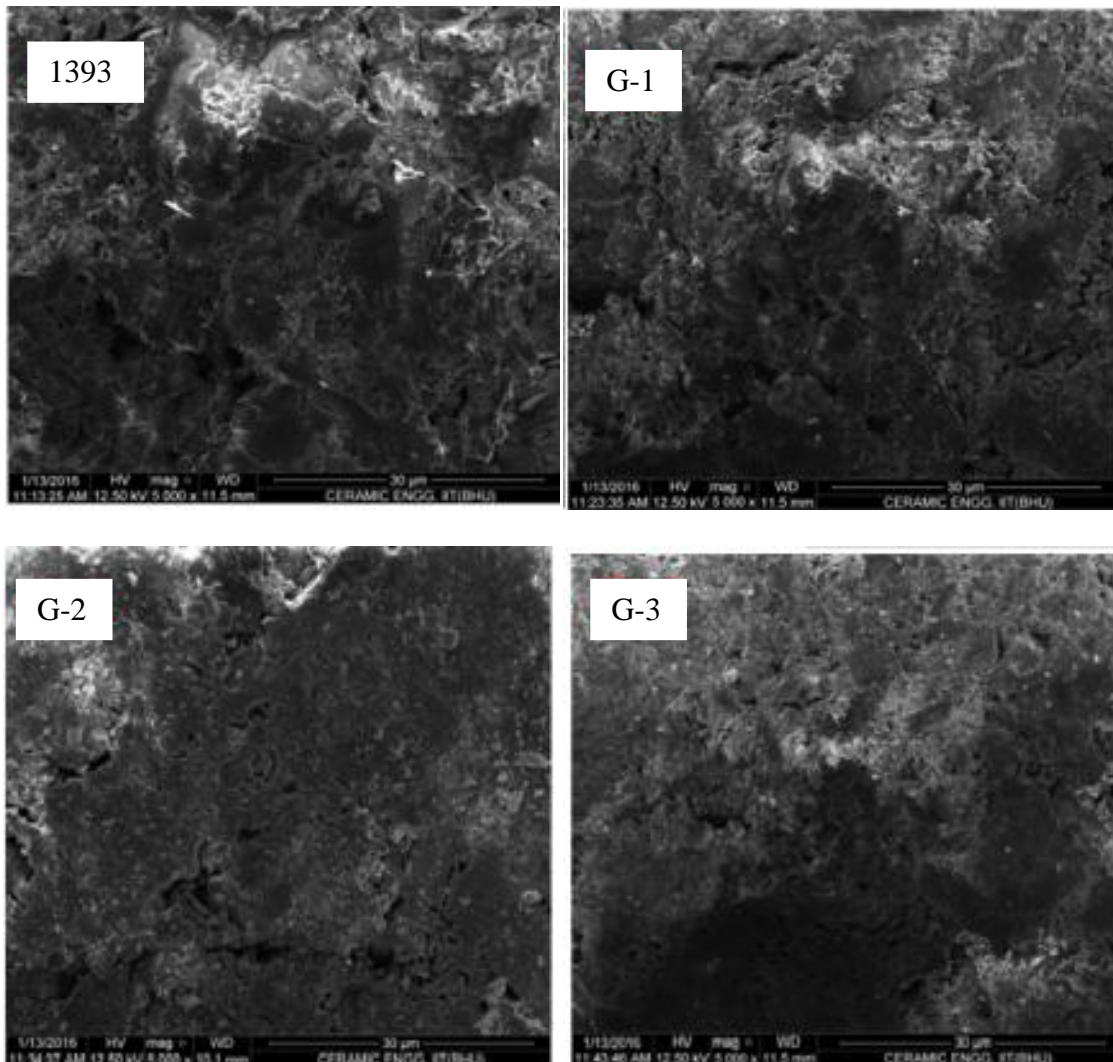


Figure 6.10- Density of 1393 bioactive glass and ZrO<sub>2</sub> doped bioactive glasses

### 6.3.7 Surface morphology by SEM

**Figure.6.11** and **Figure.5.12** show the surface morphology of base glass (1393) and the bioactive glass samples (G-1, G-2, G-3, and G-4) 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 has shown uniform polycrystalline particle on the surfaces of the glass. These developed crystals on the surface of the glasses are assumed to be HCA [ElBatal H.; Khalil, E.M.; Soltan Monem A.; Hamdy, Y.; 2003]. It is observed that for increasing  $ZrO_2$  content, the crystalline phase formed is getting uniform shapes on the glass surfaces and for G-4 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 into SBF solution. Previously It was already found out from the XRD results and FTIR spectrometry that, the HA layer is getting formed after immersion of glass into SBF for 15 days.



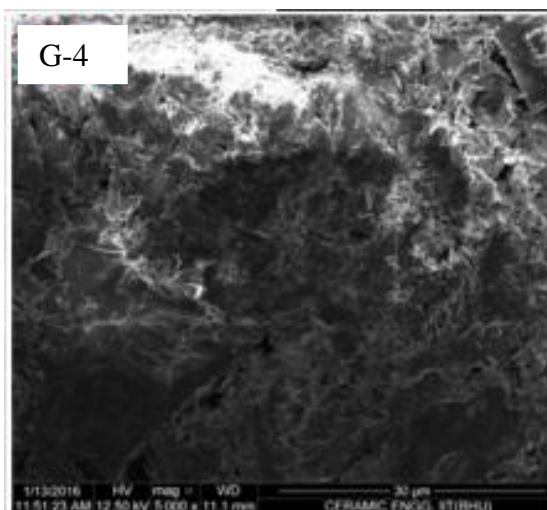
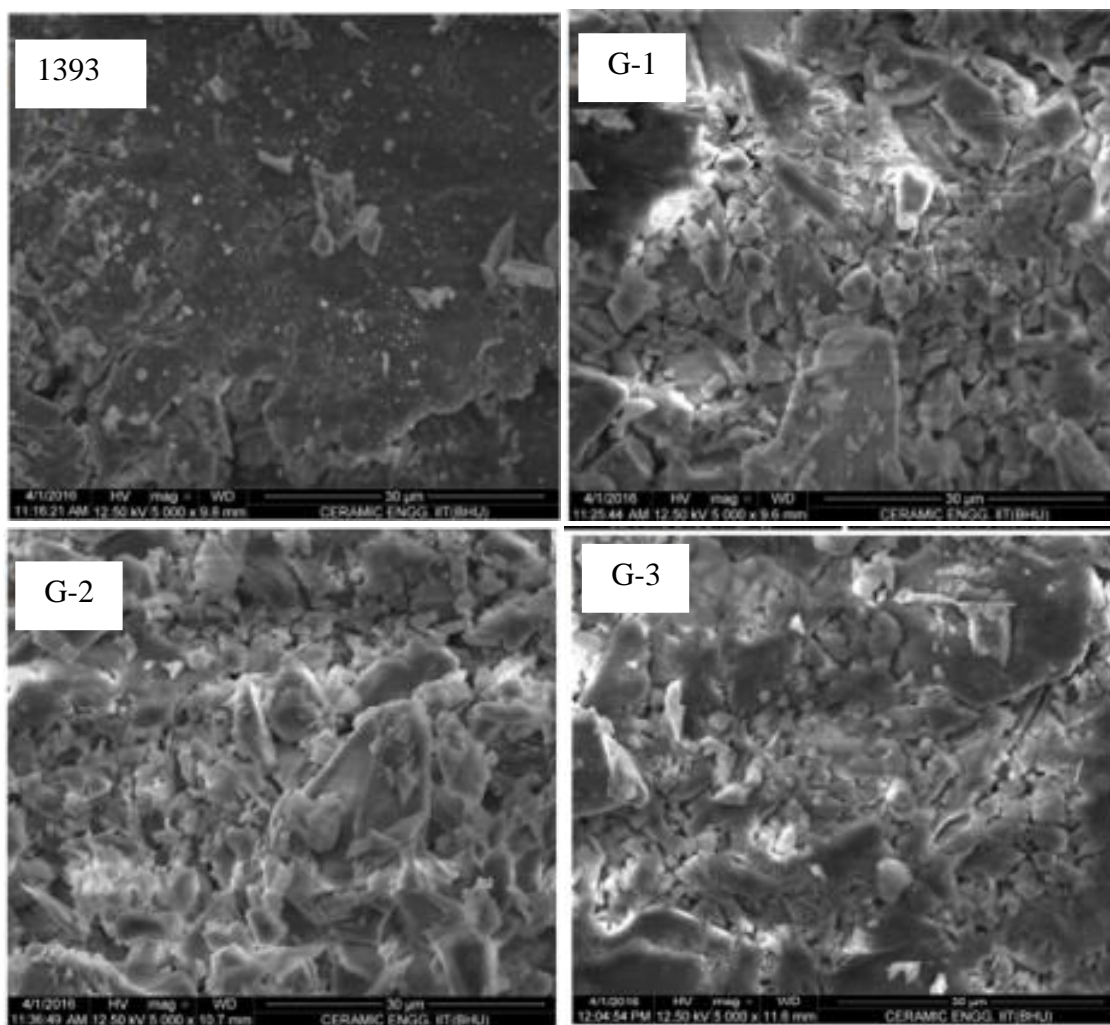
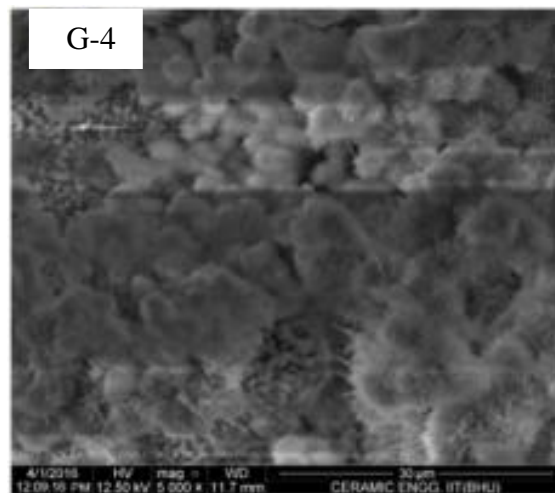


Figure 6.11- SEM images of bioactive glasses before putting into SBF solution





6.12- SEM images of bioactive glasses after putting into SBF solution

#### 6.4 CONCLUSIONS

The XRD analysis showed the amorphous nature of the glass and FTIR absorbance spectra, pH behavior; 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 the formation of HCA layer. It can be observed from the FTIR diagram of G-3 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 for bone tissue engineering applications.