

5.1 Introduction

Hench glass 45S5 [L.L. Hench et al. 1971] has been extensively used to repair hard and soft tissue bone because of their excellent bioactive properties. However, an incomplete conversion into a bone-like material undergoes by these bioactive materials which severely limits their use in biomedical application [L.L. Hench et al. 1991]. 45S5 Bioglass is generally used in making various biomedical devices, such as middle ear, dental implants, etc. Due to the brittleness nature of 45S5 bio glass and relatively its low strength, the application in non-load bearing situations is limited [W. Cao et al. 1996]. This silicate-based bioactive glass has very low degradation and remains in our body approximately more than one year after implantation [M. Hamadouche et al. 2001]. The bioglass (45S5) does not alone provide sufficient bioactivity and mechanical strength due to this reason we need to reinforce this bioactive glass with increasing content of HA and ZrO_2 .

The chemical composition of HA has similar to the inorganic mineral of our bone and teeth [AK. Nayak et al. 2010]. It has excellent biocompatibility and bioactivity. HA offers excellent biocompatibility, bioactivity, low density, low compressive strength, and low hardness. It also has relatively low mechanical properties. Therefore the use of HA as a load bearing implant teeth is very limited. Due to these limitations, there is a need for strengthening of HA without losing its biocompatibility.

It was found that zirconia (ZrO_2) possess high mechanical strength and very low toxicity. For this reason (ZrO_2) is widely used as a biomaterial for hip prosthesis, tooth crowns, dental implants and it was formed as a new bone restoring material in future. Sintered zirconia (ZrO_2) has very high mechanical strength than a cortical bone.

Therefore, new class zirconia is used as a new bone restoring material [S. Atilgan et al. 2010; H. Nisitani et al. 1994]. Because of difference in strength, the amount of stress and frequent bone fracture may occur resulting in bonding to a host bone. Due to the poor affinity to cells and tissues of zirconia (ZrO_2), there is a need to make the composite by HA and ZrO_2 . It has been observed zirconia retain high mechanical strength and toughness with HA without affecting the biocompatibility of HA. The changes in physicochemical properties of the material are due to decomposition of HA. Performance of implant material is affected by the change in its density, solubility, resorption, and biocompatibility during implantation in a living body. Therefore HA decay is the problem both from the scientific and application point of view. It has been found that calcium present in hydroxyapatite can react with ZrO_2 transformed zirconia into a cubic form which makes it tough by this transformation [J Li et al. 1993].

Further, it was observed that HA- ZrO_2 composites have the very improved strength and toughness as compared to monolithic HA itself [J Zhang et al. 2006; KA Khalil et al. 2007; A Rapacz-Kmita et al. 2006; Wu et al. 1988]. It is well known that P_2O_5 act as a strong glass network former. The covalent bond is formed between PO_4 tetrahedra structures in chains or rings by bridging oxygens [G. Little flower et al. 2007]. It is also known that Na_2O and CaO were acted as glass network modifiers and formed non-bridging oxygens (NBO) into the glass network [Reddy M. Srinivasa et al. 2008]. In environment Zr present as a common trace element or metallic Zr(IV) usually present in human bone and tissue as low in the range of 2–10 mg/kg body weight with an estimated average daily intake in humans of ~2.6 mg. The toxicity of Zr has been assessed low to moderate in animals. Ceramics can be divided into two main groups that have been used as an implant material, i.e., bioinert and bioactive. Bioinert

ceramics, such as zirconia (ZrO_2) show no interaction with the surrounding and living tissue. However, bioactive ceramics such as calcium phosphates are forming bonds with living hard and soft tissue. Since it has the main inorganic constituent of bones and teeth. HA is the most interesting bioactive ceramics [M Vallet-Regi et al. 2004].

Partially stabilized zirconia (PSZ) is bioinert and has the highest strength, and fracture strength was mixed with HA to obtained bioactive implants with increased mechanical properties. Similarly other oxides, such as alumina (Al_2O_3), titania (TiO_2) and yttria (Y_2O_3) [P. Parente et al. 2012] were studied to prepare HA composites in different structures via several methods.

5.2. Material and Methods

5.2.1 Selection of composition

The composition of bioglass and biocomposites were illustrated in Table 5.1.

Table 5.1: Composition of Bioglass and Biocomposites (BHZ1, BHZ2, BHZ3, BHZ4).

| Sample | Composition (wt %) | | | |
|-----------------------------|--------------------|--------------|------------|---------------------------|
| BG (45S5) | 45 SiO_2 | 24.5 Na_2O | 24.5 CaO | 6 P_2O_5 |
| Biocomposite Samples | BG (45S5) | HA | | ZrO_2 |
| BHZ1 | 85 | 10 | | 5 |
| BHZ2 | 70 | 20 | | 10 |
| BHZ3 | 55 | 30 | | 15 |
| BHZ4 | 40 | 40 | | 20 |

5.2.2 Synthesis of BG-HA-ZrO₂ composites

45S5 Bioglass was prepared by using analytical grade quartz, calcium carbonate, sodium carbonate, and ammonium dihydrogen orthophosphate by melting at 1400-1410°C with air as an atmosphere and annealed in an air oven at 500-550°C. Hydroxyapatite was prepared by sol-gel technique Fig.2.3. Bioglass (45S5), HA and ZrO₂ powder were milled and mixed by ball milling about 4 hours. Samples were placed and sintered in a furnace at 1000°C for 5 hours, the rate of heating and cooling is at 5°C/minute. The uniaxial pressure of 100 MPa was applied to form a rectangular bar shape sample of 4 mm×6 mm×40 mm size by using a die of size 55 mm×10 mm. The composition of biocomposites was illustrated in Table 5.1.

5.3. Results and discussion

5.3.1 XRD analysis of biocomposite

The XRD pattern of sample BHZ1, BHZ2, BHZ3, BHZ4 has presented in Fig.5.1, was heated at 1000°C contained hydroxyapatite and tetragonal-zirconia phases. Upon heat treatment at higher temperatures, i.e., 1100°C and 1200°C, the zirconia and wollastonite gradually transformed into zirconium silicate (zircon) respectively. Before and after soaking in SBF for various time periods i.e.1, 3, 7, 14 and 21 days, typical XRD patterns were obtained on the surfaces of biocomposites. XRD patterns of the biocomposite sample is shown in Fig.2(a-d). After immersion in SBF, crystalline peaks appear in the XRD patterns which indicates the formation of a crystalline layer on the surface of the biocomposites. Initially, well-defined hydroxyapatite (HA) peaks develop at (2θ) values of 23-27° after three days of soaking in SBF and ZrO₂ peaks develop at (2θ) values of 30-35° after seven days of immersion.

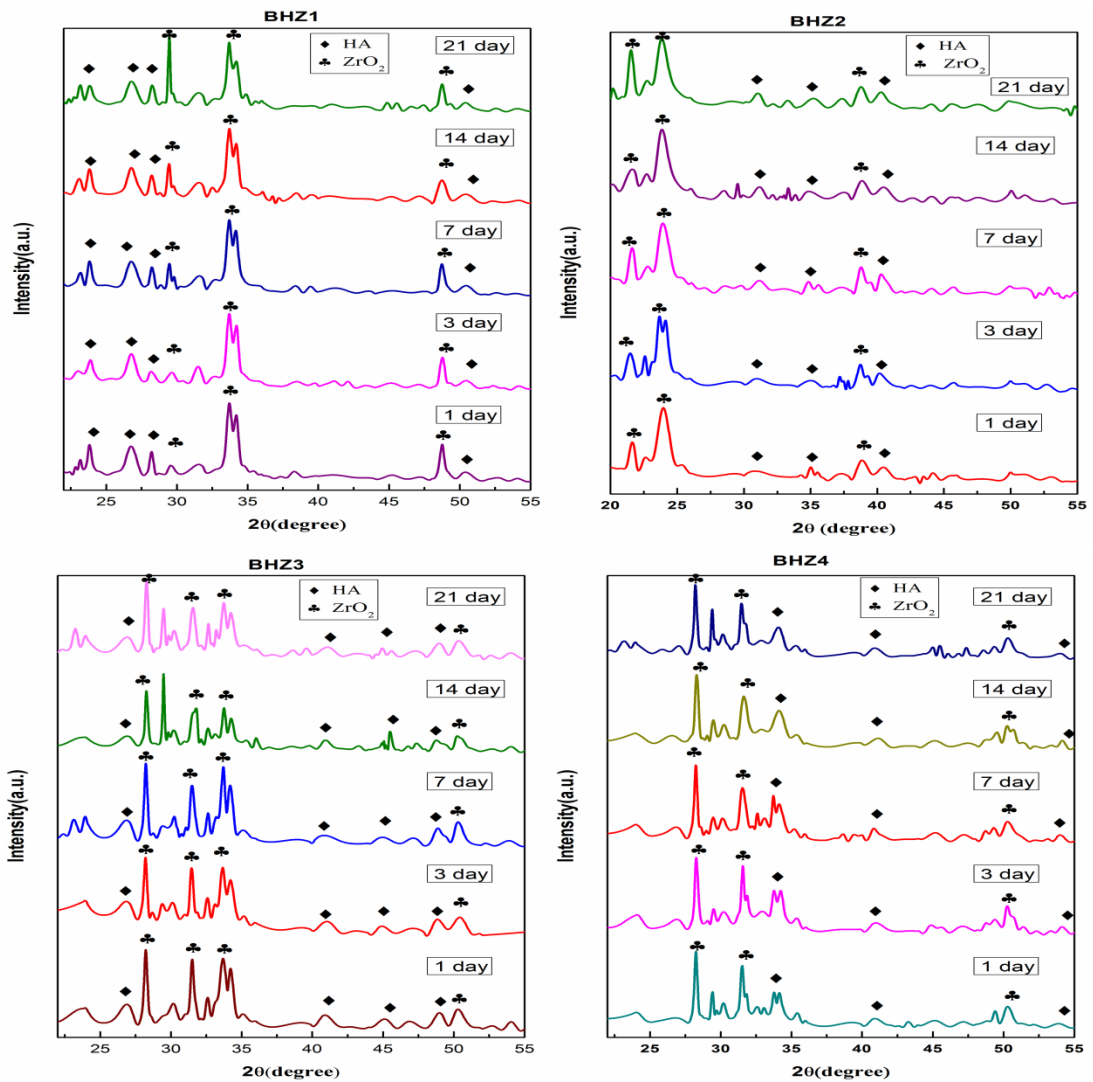


Figure 5.1 XRD pattern of biocomposite sample BHZ1, BHZ2, BHZ3 and BHZ4 after SBF treatment (1,3,7,14,21) days.

The reaction between the HA and the ZrO_2 forms TCP and cubic ZrO_2 as follows [Wu et al. 1988]:



Hydroxyapatite transforms into TCP by releasing calcium (or CaO) and water vapor as shown in equation (5.1). HA phase is not stable, and TCP is a major phase. According to the reaction (5.2), a lot of CaO is released. Thus cubic-ZrO₂ are formed.

5.3.2 Transmission FTIR analysis of biocomposite

FTIR spectra bands of BHZ1 sample illustrated in Fig.5.2 for 3,7,14 and 21 days treated with SBF. At 516 and 639 cm⁻¹, P–O bending (crystalline) and P–O bending (amorphous) was formed. At 916 cm⁻¹ C–O stretching band shown which indicates the formation of HCA layer. The bands at about 1512 and 1695 cm⁻¹ were related to C–O (Stretch) and C=O (Stretch) modes respectively, and the broad band at about 3748 cm⁻¹ are formed due to O–H groups on the surface. The samples in SBF for prolonged period indicates the similar response with a small decrease in the intensities of the bands due to the formation of HCA layer. Further, almost similar vibrations in all specimens (BHZ2, BHZ3, BHZ4) in Fig.3(b),(c),(d) which confirm apatite formation have also shown in table 5.2.

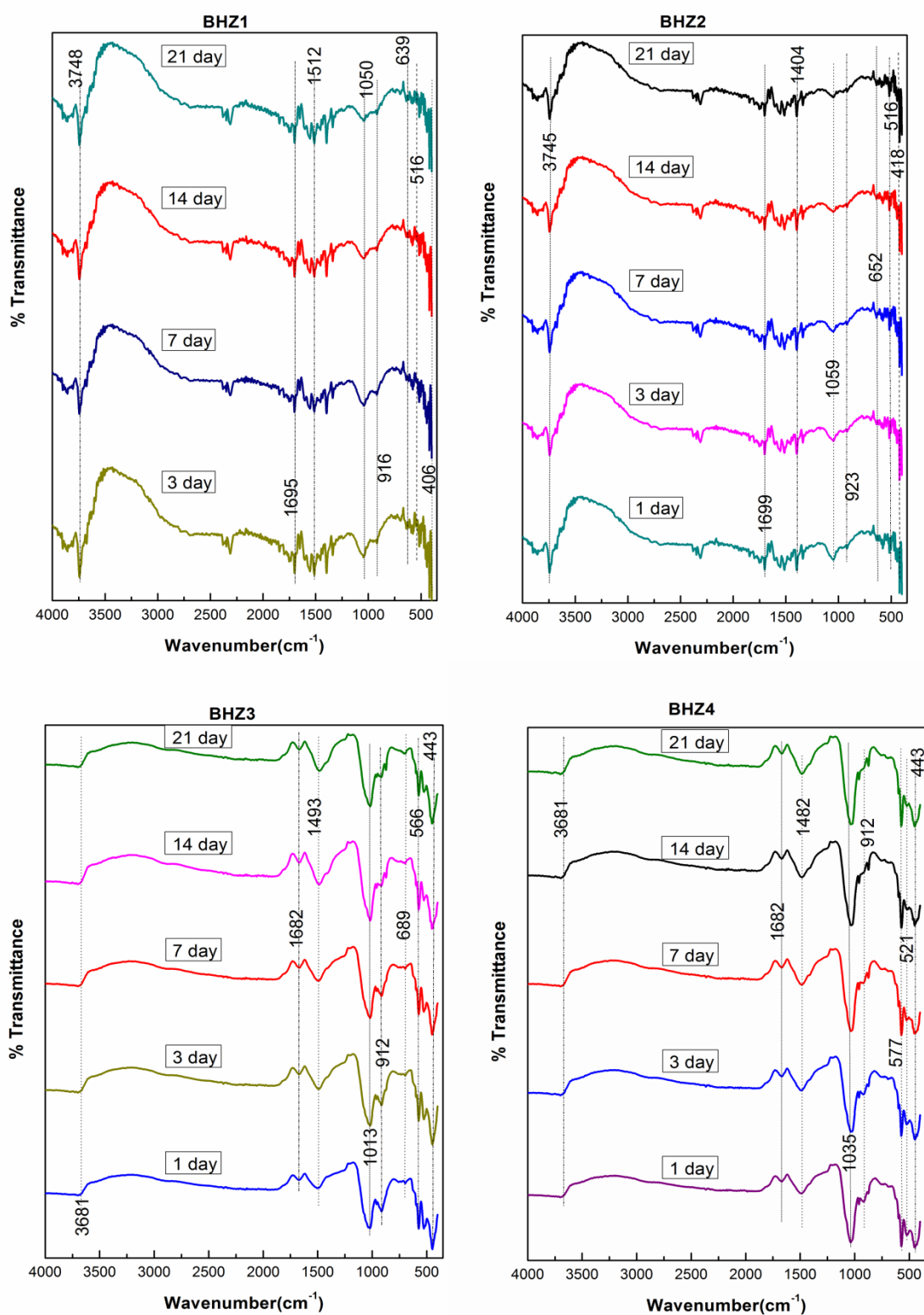


Figure 5.2 FTIR of the biocomposite sample BHZ1, BHZ2, BHZ3 and BHZ4 after immersion in SBF treatment for (1,3,7,14,21) days.

Table 5.2 : FTIR spectra bands of BHZ1, BHZ2, BHZ3, BHZ4 sample.

| Sample | P–O (crystalline) | P–O (amorphous) | C–O (Stretch) | C=O (Stretch) | O–H groups |
|--------|----------------------|--------------------|------------------|------------------|------------|
| BHZ1 | 516 | 639 | 916, 1512 | 1695 | 3748 |
| BHZ2 | 516 | 652 | 923, 1404 | 1699 | 3745 |
| BHZ3 | 566 | 689 | 912, 1493 | 1682 | 3681 |
| BHZ4 | 521 | 577 | 912, 1482 | 1682 | 3681 |

The samples in SBF for prolonged period shows the same response with small decrease in the intensities of the bands that resulted in the formation of HCA layer.

Zr can be react in two ways.

1. Zr as glass network modifier: Possibility of some splitting in Si-O-Si bonds leads to the lower connectivity of the silicate network with the increase in O/Si ratio in BHZ concerning 45S5. This phenomenon results in the decrease of bridging oxygens and increase in non-bridging oxygens. However, after the incorporation of Zr, we found a significant reduction in the number of non-bridging oxygen in the FTIR spectra of the dried gels.

2. Zr as glass former: Zr may even act as a glass former with $[\text{ZrO}_4]^{4-}$ units occupying some positions of $[\text{SiO}_4]^{4-}$ tetrahedra. The extent of shifting the ^{29}Si , resonance for a given tetrahedron is positive due to each covalent Si–O–Zr bridge [L. Barbieri et al. 2003].

5.3.3 pH analysis of biocomposite samples

Change in pH of biocomposite (BHZ1, BHZ2, BHZ3 and BHZ4) are presented in Fig.5.3 after immersing all samples in SBF for the different time. It is found that pH has increased up to 7 days due to the fast release of alkali ions (Na^+) and alkaline earth ions (Ca^{2+}) and its exchange with H^+ or H_3O^+ ions in the simulated body fluid (SBF) solution. There is an increase in OH^- ions which increase the pH of the solution and breaking of Si-O-Si bonds which hold the glass structure together. Thus there is a formation of silanols which decrease the pH of the solution after 7 to 21 days. Soaking in SBF leads to the creation of an apatite layer on the surface of the composite samples depends upon the morphological properties of the biocomposite sample [H Satoshi et al. 1999; K. Toshihiro et al. 2001]. In vitro hydroxyapatite formation on body fluid inside simulated body fluid (SBF) [AE Clark et al. 1976] has four proposed stages. First: Fast cation exchange of Na^+ and Ca^{2+} with H^+ in solution, forming silanol bonds (Si-OH) on the glass surface, i.e., $\text{Si-O-Na}^+ + \text{H}^+ + \text{OH}^- \rightarrow \text{Si-OH} + \text{Na}^+(\text{aq}) + \text{OH}^-$, Second: A silica-rich (cation vacant) regions forms near the glass surface results increase in pH. Phosphate is also dissipated from the glass is available in the composition. Third: High local pH splits the Si-O-Si bonds and releases OH^- which causes an attack to the silica glass network. Soluble silica dissolves in the solution in the form of $\text{Si}(\text{OH})_4$ and leaving more Si-OH (silanols) at the glass-solution interface: $\text{Si-O-Si} + \text{H}_2\text{O} \rightarrow \text{Si-OH} + \text{OH-Si}$, Fourth: Condensation of Si-OH groups near the glass surface due to re-polymerization of the silica-rich layer.

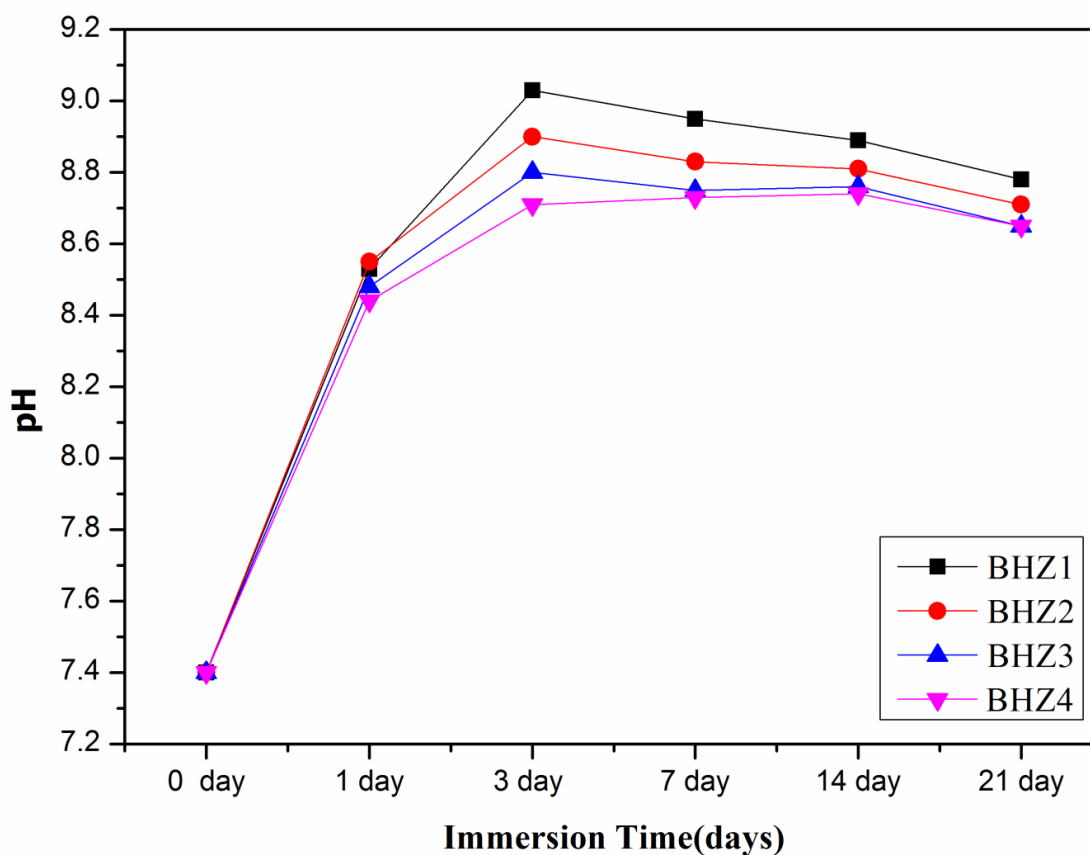
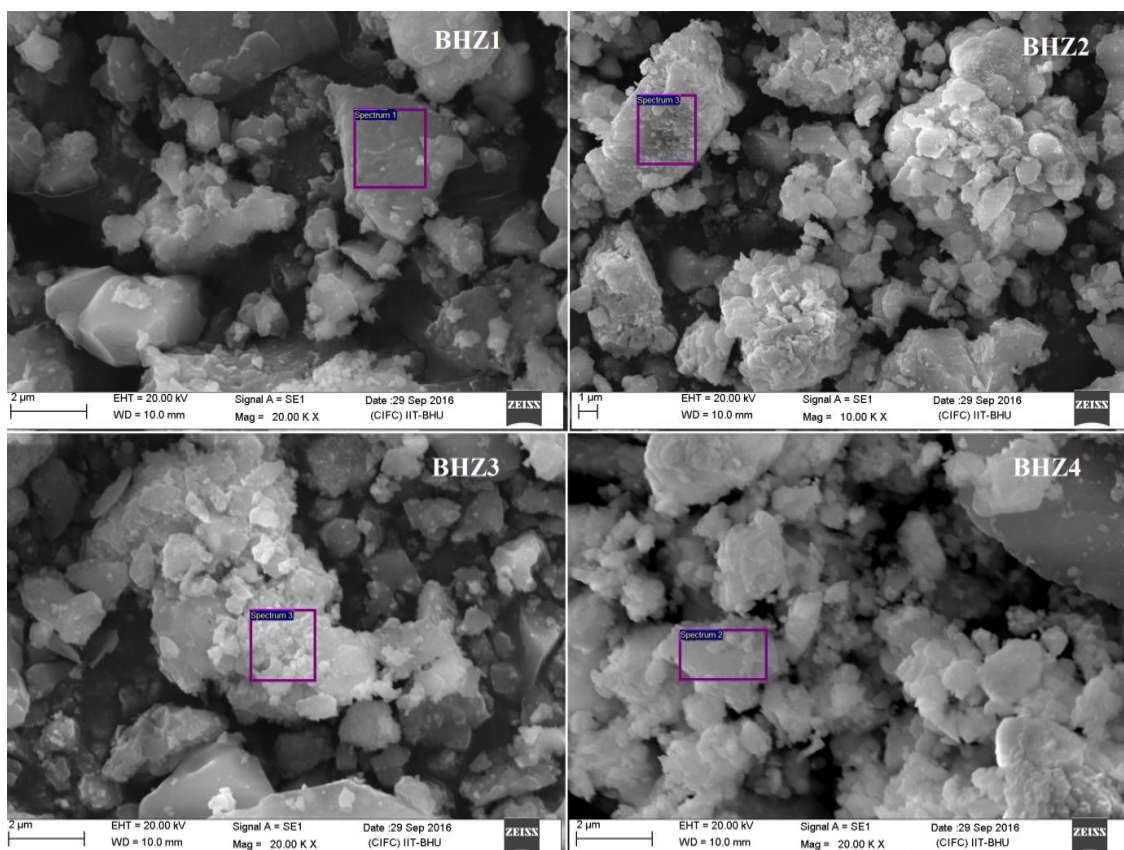


Figure 5.3 pH behaviour of the SBF after immersion of the biocomposite samples (BHZ1, BHZ2, BHZ3, BHZ4).

5.3.4 SEM and EDS analysis of biocomposite sample after soaking in SBF

After in vitro studies, the surface morphology and hydroxyapatite layer formation on the surface of BHZ samples are shown using (SEM–EV018, CarlZeiss, UK) Fig.5.4. Before in vitro studies, a compact and uniform morphology is viewed for BHZ sample. Where BHZ samples obtained after 21 days of immersion in SBF, clear spherical apatite crystals have seen on the surface, which matches with the measured results[P. Li Ohtuki C. et al. 1993]. Due to the addition of hydroxyapatite and ZrO_2 in BHZ sample a stable formation of HA layer on the sample surface took place after in vitro studies. The

above results show that the reactivity of BHZ sample is high in SBF samples. Fig. 5 shows the cross-sectional view and corresponding energy dispersive (EDS) spectra of BHZ sample after in vitro studies by using an instrument (EDS-51N1000, Oxford, UK). The observed results show an embedded spherical hydroxyapatite layer on the surface of the biocomposite. Calcium and phosphate on the biocomposite surface are confirmed by EDS spectra of BHZ sample, which shows the presence of strong bioactivity.



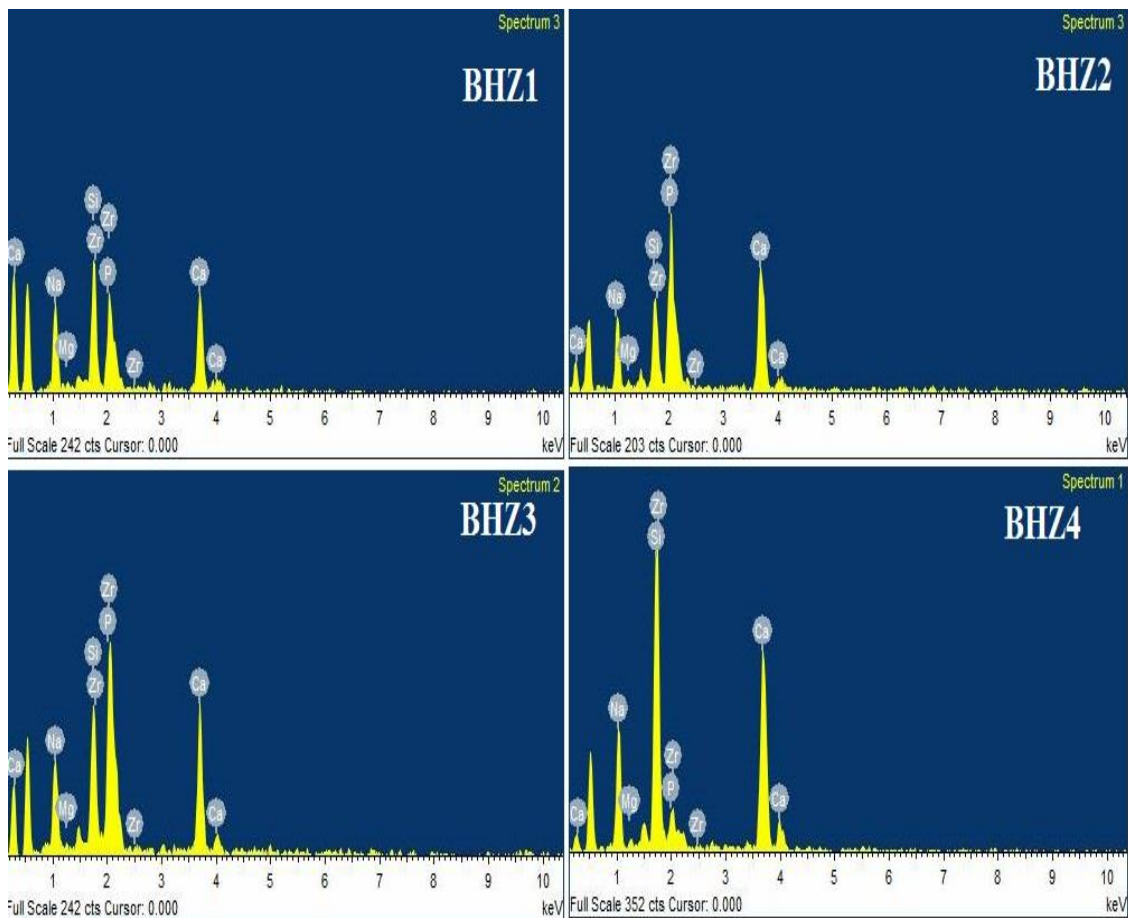
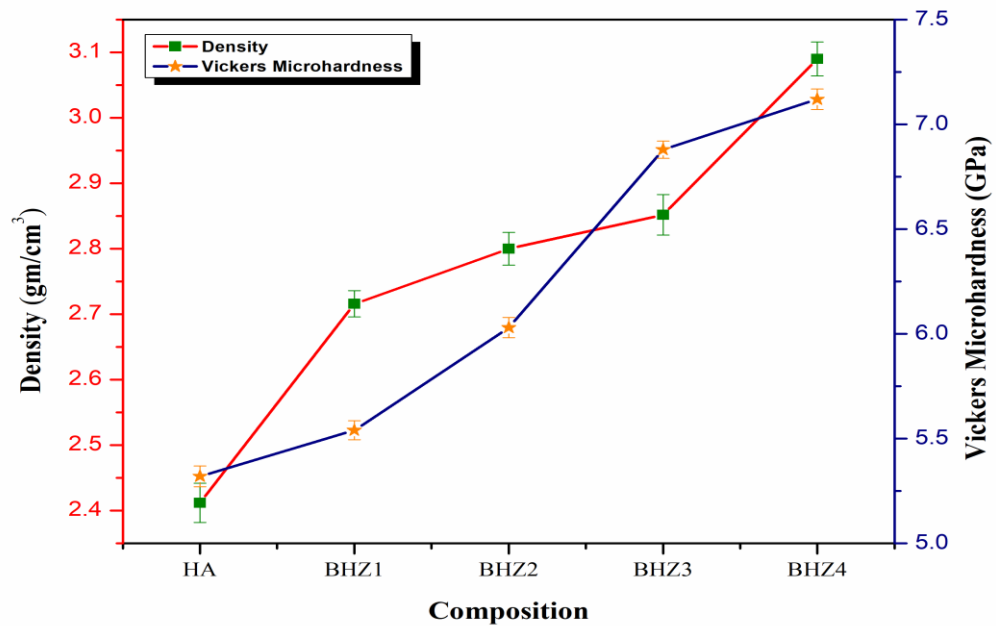


Figure 5.4 SEM of biocomposite samples after SBF and EDX of biocomposite samples.

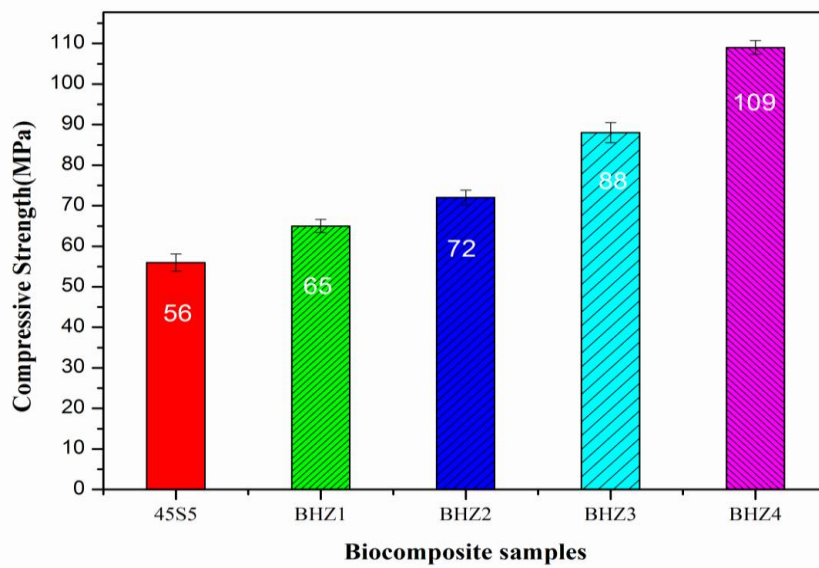
5.3.5 Mechanical properties of BHZ biocomposites

Fig.5.5(a) shows the density and microhardness of biocomposite, and this result shows an increase in hydroxyapatite and zirconia content which leads to increase in density and hardness value. In Fig.5.5(b) the strength of the 45S5, BHZ1, BHZ2, BHZ3, BHZ4 composite samples was about 56MPa, 65MPa, 72MPa, 88MPa,109MPa respectively and result depicts an increase in strength of BHZ biocomposite with increasing content of HA and ZrO₂ reinforcement. The fracture toughness of the BHZ1, BHZ2, BHZ3, BHZ4 biocomposite samples were 0.672, 0.691, 0.699, 0.715 MPa.m^{1/2} respectively.

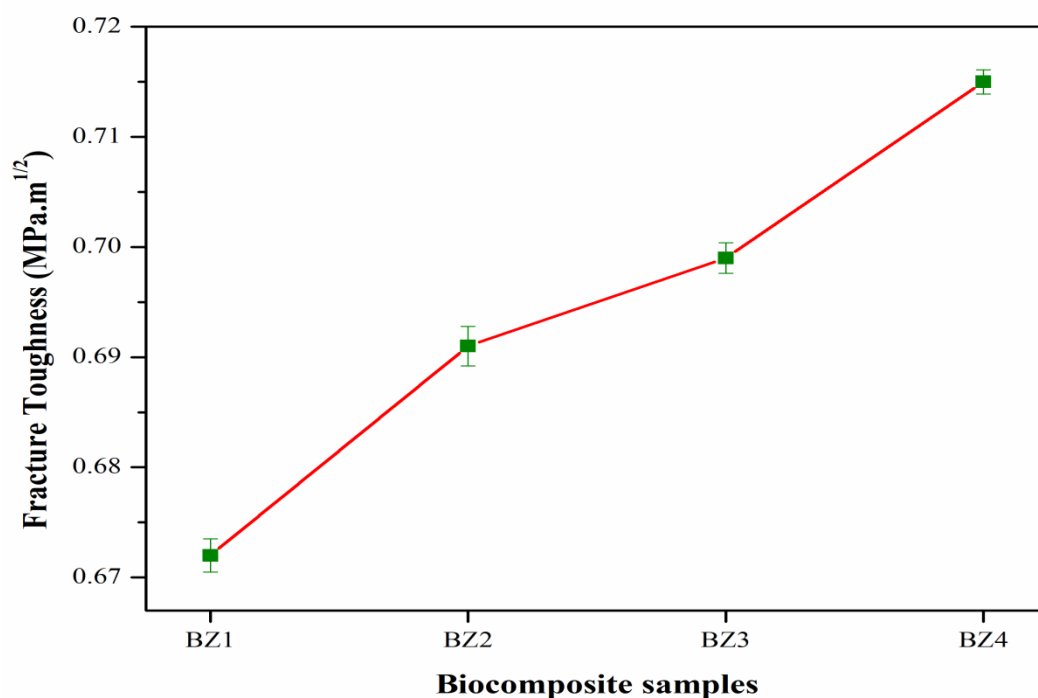
In Fig. 5.5(c) result shows an increase in fracture toughness with increasing reinforcement of HA and ZrO₂ in base 45S5 bioglass.



(a)



(b)



(c)

Figure 5.5 (a) Density and Vickers microhardness (b) Compressive strength (c) Fracture toughness of biocomposites (BHZ1, BHZ2, BHZ3, BHZ4) sample.

5.4. Conclusions

The increasing amount of hydroxyapatite (10,20,30,40wt%) and zirconia (5,10,15,20 wt%) reinforced with base bioactive glass up to the limit increase the bioactivity. Beyond 20wt%, the addition of ZrO₂ causes a decrease in maxima of the pH of SBF solution containing immersed samples. In the present investigation, it was found that biocomposite has 40wt% HA, 20wt% ZrO₂ and 40wt% bioactive glass have the highest bioactivity, density, microhardness, compressive strength and fracture toughness. Thus, we can say that the reinforcement of HA+ZrO₂ in the 45S5 bioactive glass would be a good bioactive material.
