3.1. Introduction

45S5 Bioglass® is a class of bioactive glass capable of naturally bonding to human bone and soft tissue in physiological media [L. L. Hench et al. 1971; L. L. Hench et al. 1973; J. Wilson et al. 1981]. Hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$, HA) has attracted much attention as a material for artificial bones. Hydroxyapatite has been widely used as a bone substitute material in restorative dental and orthopedic implants, due to its chemical and crystallographic structure being similar to that of bone mineral. Calcium phosphate ceramics, especially HA are currently used as biomaterials for many applications in both dentistry and orthopedics [F.J. Garcı´a-Sanz et al. 1997].

3.2. Material and Methods

3.2.1.Preparation of Bioglass

The bioactive glass composition was formulated from Na_2O-CaO-SiO_2-P_2O_5 glass system. Proposed bioactive glasses containing chemical composition $45SiO_2$-$24.5Na_2O$-$24.5CaO$-$6P_2O_5$ was prepared. The compositions of prepared bioactive glasses are given in table 3.1. The bioactive base glass was prepared by the normal melting and annealing technique. Fine-grained materials were used for quartz. Lime and soda were introduced in the form of their respective anhydrous carbonates. P_2O_5 was added in the form of ammonium dihydrogen phosphate. The weighed batches were mixed thoroughly for 30 minutes and melted in 100 ml high alumina crucibles to get the desired bioactive glass. The melting was carried out in an electric furnace at 1400±10°C for 4 hours in the air as furnace atmosphere and homogenized melts were poured on the preheated aluminum sheet. The prepared bioactive glass samples were directly transferred to a regulated

muffle furnace at 470°C for annealing. After 1 hour of annealing, the muffle furnace was cooled to room temperature with the controlled rate of cooling at 20°C per hr.

3.2.2 Production of HA

In the present study, calcium nitrate tetrahydrate ($Ca(NO_3)_2 \cdot 4H_2O$) (CNT) (G.S. Chemical Testing Lab. & allied industries, India), phosphoric acid (H_3PO_4) (Loba Chemie Pvt. Ltd, India) and ammonia (NH_3) (Loba Chemie Pvt. Ltd, India) were used as initial precursors. The schematic presentation of the procedure [K. P. Santosh et al. 2009] is given in Figure3.1.

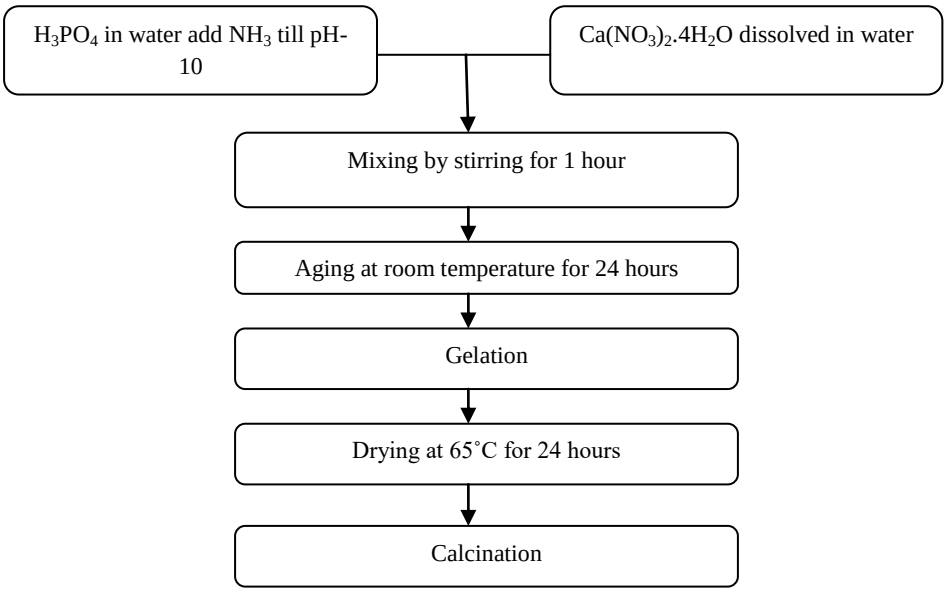


Figure 3.1 Flow chart of hydroxyapatite preparation by the sol-gel route.

Table 3.1: Composition of Bioglass and Bio-composite (BC1, BC2, BC3, BC4).

	Composition (wt %)			
BG (45S5)	45 SiO_2	24.5 Na_2O	24.5 CaO	6 P_2O_5
Biocomposite Samples	**BG (45S5)**		**HA**	
BC1	95		5	
BC2	90		10	
BC3	85		15	
BC4	80		20	

3.2.3 Preparation of BG/HA composites

By melting route, bioglass powder is mixed with HA powder (sol-gel) 5,10,15,20 (wt%) compacted at 1500 MPa pressure into cylindrical samples (1 cm,1 cm) and sintered at 1000-1050ºC to prepare the composites as shown in Table 3.1.

3.3. Results and discussion

3.3.1. Differential Thermal Analysis (DTA/TGA) of Bioactive Glass composite

The differential thermal analysis (DTA/TGA) curve of biocomposite shown in Fig.3.2. Due to the incorporation of hydroxyapatite in the base bioactive glass, there is an increase in glass endothermic as well as exothermic peaks. This increase in temperature is due to hydroxyapatite acting as a modifier which strengthens the (Si-O-Si) silica network. The results demonstrated the Tg temperature was from 489°C to 554°C and Tc from 1005°C to 1093°C [M. M. Azevedo et al. 2010].

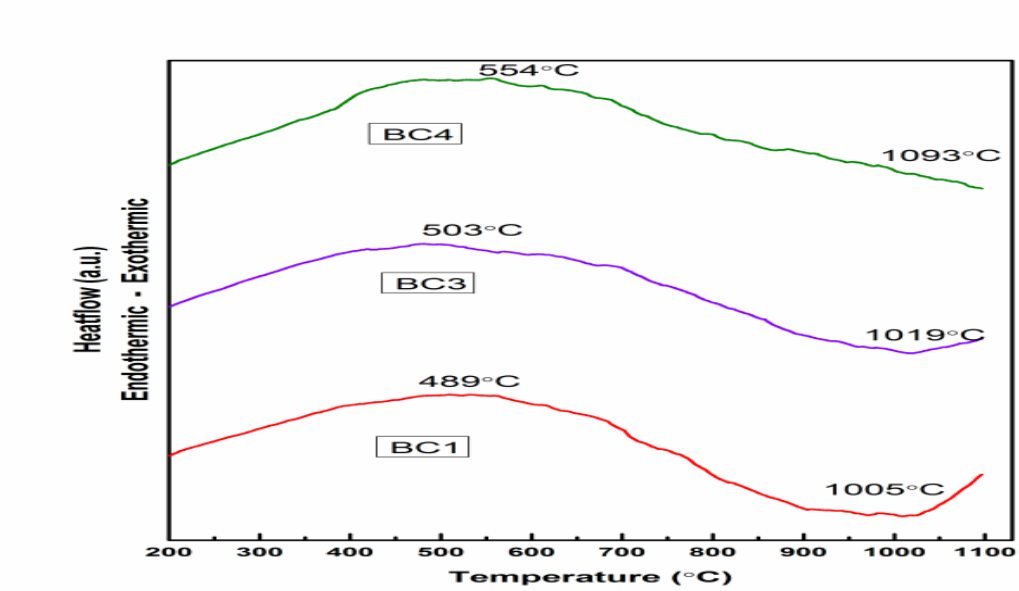


Figure 3.2 DTA/TGA analysis of bioglass based biocomposites.

3.3.2. Phase analysis:

XRD was used to characterize the prepared samples of bioglass/HA composite as BC1, BC2, BC3 and BC4 (all of the samples in Table 3.1). XRD patterns of these samples show that the main phases are pseudo wollastonite and hydroxyapatite (JCPDS No.: 090432). The conclusion indicates that the synthesized bioglass has been crystallized at 1000˚C. It has been reported that pseudo wollastonite is a bioactive material, and its in-vitro, in-vivo tests have been investigated [A.J.Salinas et al. 2002; P.N. De Aza et al. 2004; C.Sarmento et al. 2004; P.N. De Aza et al. 2000; P.N. De Aza et al. 1999].

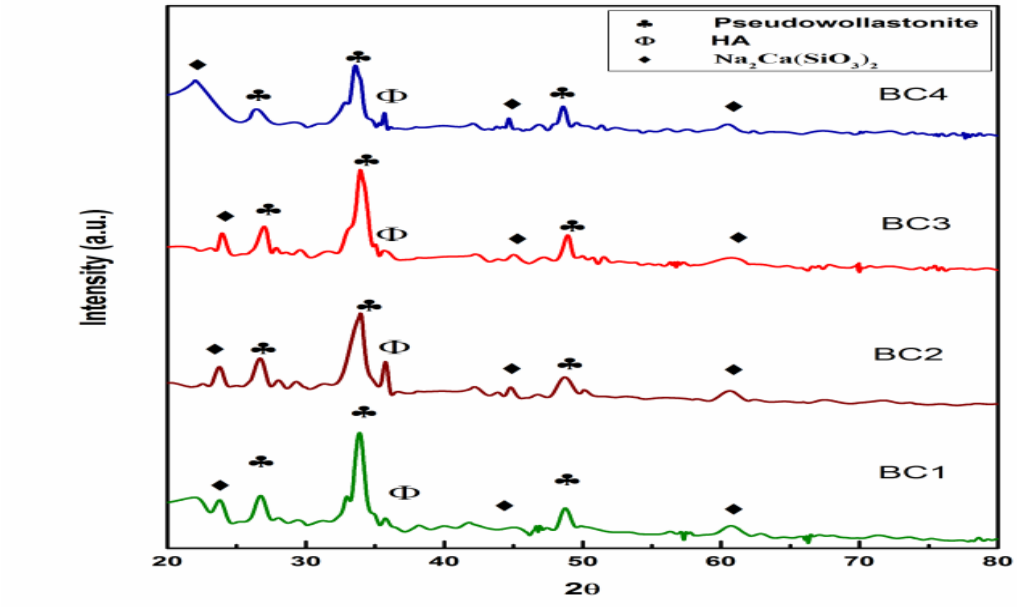


Figure 3.3 X-ray diffraction of the prepared (BC1,BC2,BC3,BC4) composites.

To compare the intensity of the formed phases results can be seen in Fig.3.3 two characteristic peaks of pseudo wollastonite (JCPDS No.:19-0248) and hydroxyapatite was selected for comparison. 2θ = 36.80 for Wollastonite and 2θ = 40.17 for hydroxyapatite phase were selected, and their intensities were compared. The silicon and sodium oxides could react with hydroxyapatite phase in biocomposite as a result, the OH ions would be eliminated from the structure as water vapor. This reaction will cause the formation of sodium-calcium silicates ($Na_2CaSi_2O_6$), which was sintered at 1000°C mainly shows the presence of sodium-calcium silicates ($Na_2CaSi_2O_6$, JCPDS # 77-2189). It indicates that sintering promotes the transformation of hydroxyapatite to β –TCP [M. M. Sebdani et al. 2012].

$$2Ca_5(PO_4)_3OH + Na_2O + 2SiO_2 \longrightarrow Na_2CaSi_2O_6 + 3Ca_3(PO_4)_2 + H_2O \quad ---(3.1)$$

It has been found that sample BC4 has the most content of hydroxyapatite and sample BC1 has the least content of hydroxyapatite thus it can be said that it has the most content of wollastonite.

3.3.3. SEM analysis of bioactive biocomposite samples

Surface morphology after post-immersion in SBF the glass releases Ca^{2+} and Na^{+} ions from its surface via an exchange with the H_3O^{+} ion in the SBF to form Si–OH groups on their surfaces [L.L. Hench et al. 2001]. Water molecules in the SBF simultaneously react with the Si–O–Si bond to form additional Si–OH groups, the formed Si–OH groups induce apatite nucleation, and the released Ca^{2+} and Na^{+} ions accelerate apatite nucleation by increasing the ionic activity product of apatite in the fluid [T. Kokubo et al. 2003]. As a result, after soaking in SBF in a short period (3days), the apatite layer forms onto the composite surface, and this phenomenon is confirmed by SEM of BG/HA composites post-immersion as shown in Fig. 3.4(a-d). BC1 biocomposites show that composite has many particles on its surface proving slight formation of apatite layer because it contains the high content of silica characterizing melted and compact structure that reduces nucleation of apatite layer as compared to other composites. The formation of silanol groups on the material's surface which are essential for nucleation sites for HA formation is due to the simultaneous dissolution of silicates [K.H. Karlsson et al. 2004]. Once the apatite nuclei formed, they can grow spontaneously by uptaking calcium phosphate ions from the surrounding fluid [Y. Ebisawa et al. 1993]. For BC2, BC3, BC4 composites, The SEM at the same magnification indicate the presence of rich spherical shapes build upon each other to

form a bone-like apatite layer for both composites especially BC4 composites.

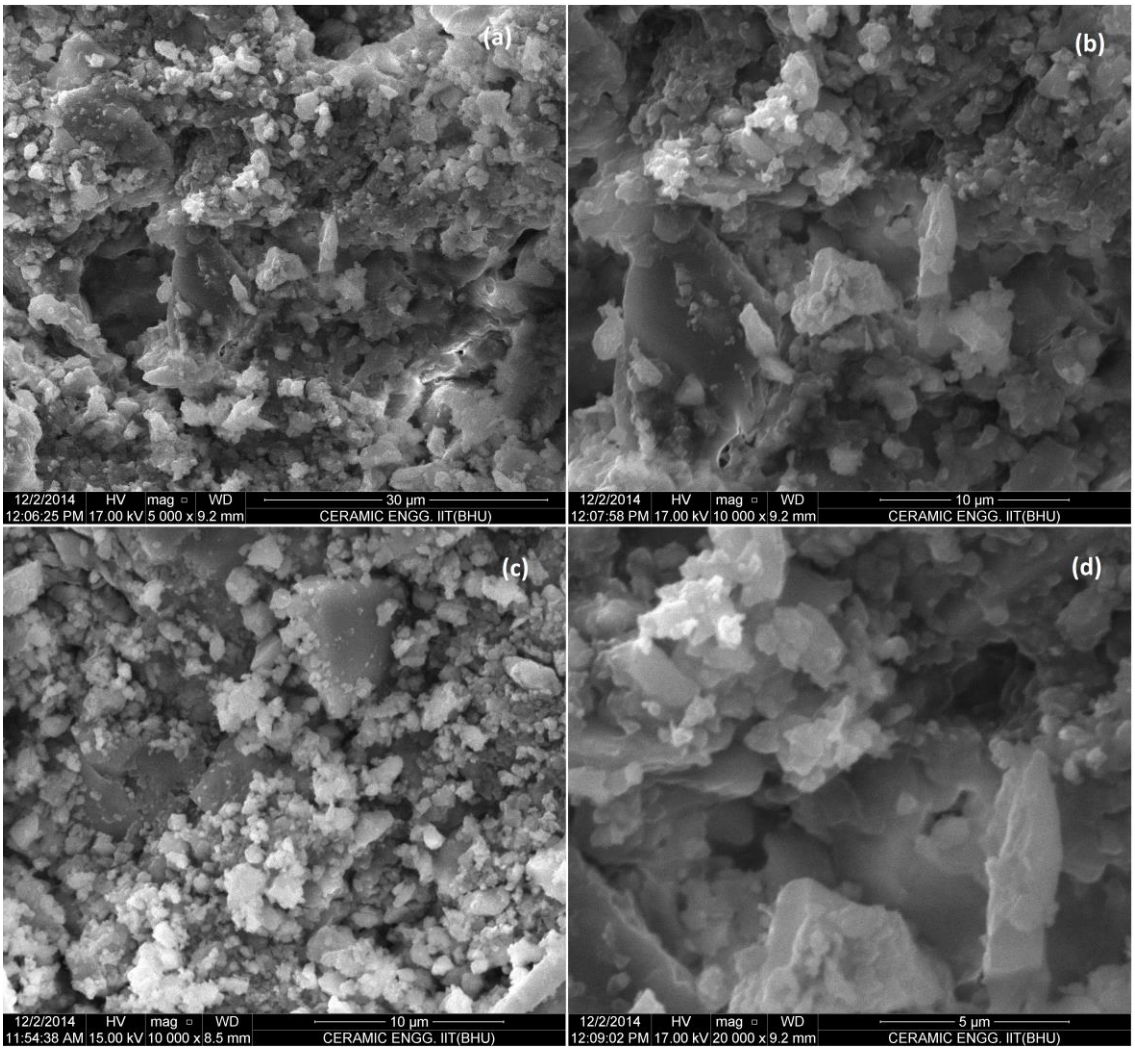


Figure 3.4 (a-d) SEM micrographs of bioactive composites samples (BC1,BC2,BC3,BC4) which were soaked in SBF solution for 30 days.

3.3.4 pH behavior in SBF

After soaking of biocomposite for various time periods, the variation in pH values of simulated body fluid (SBF) is shown in Fig.3.5. It was observed that the pH of all samples shows the similar trend of behavior [Cerrutia Marta et al. 2005]. On one day of immersion, maximum pH values were recorded. Ion exchange method was used to explain, the change in pH of SBF solution on the glass surface. Cations such as Na^+ or Ca^{2+} near the glass surface releases into the solution in exchange of H^+ or H_3O^+ ions from the solutions which result in pH increase. After the specific point, the precipitation of calcium phosphates and carbonates results in the decrease in pH. The update of carbonate and phosphate ions shifts the equilibriums towards the products side and causes the decrease in the pH [S. Brauer Delia et al. 2010]. There is an addition of HA in base bioactive glass (45S5) to make biocomposites. After immersion of biocomposite in SBF for various time periods, the chain of reactions occurs in the solution which favors the formation of hydroxyapatite layer on the surface of the samples [V. R. Mastelaro et al. 2000; P. Ducheyne et al. 1999].

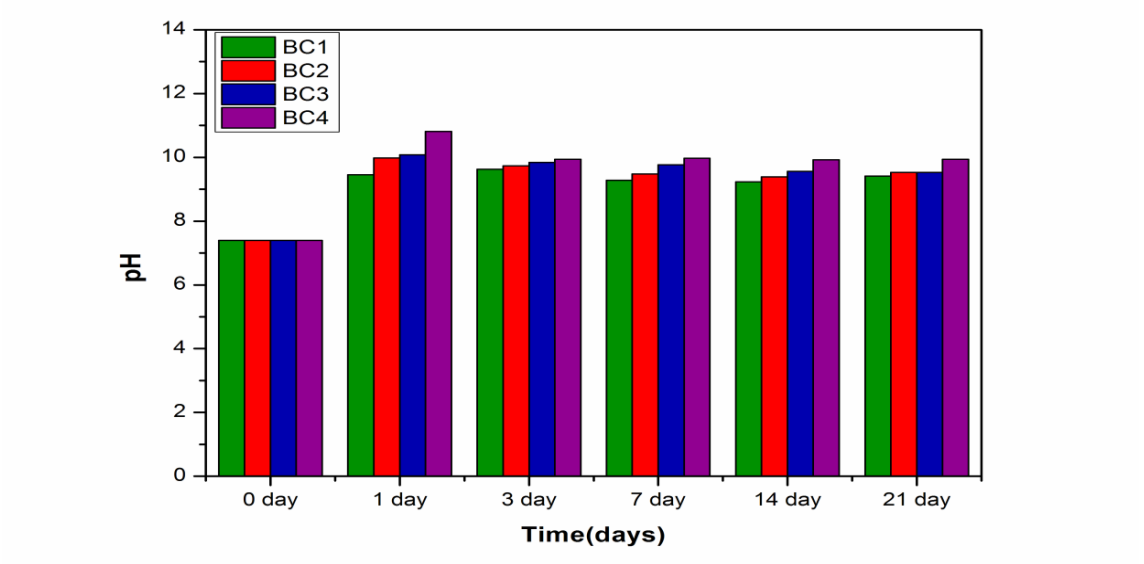


Figure 3.5 pH of different biocomposite (BC1,BC2,BC3,BC4).

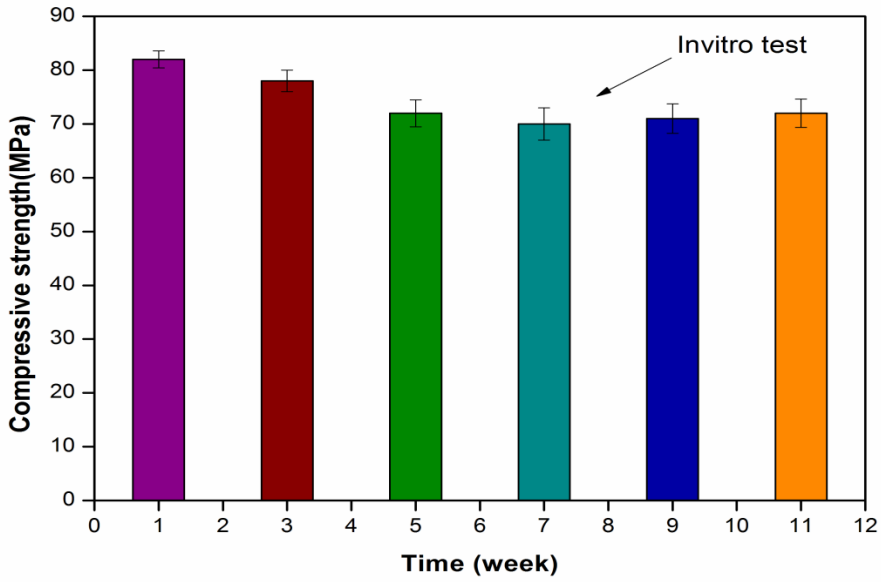
3.3.5 Mechanical Properties

Table 3.2: Density (ρ), longitudinal velocity (V_L), transverse velocity (V_T), young's modulus, shear modulus, bulk modulus and poisson's ratio of biocomposites.

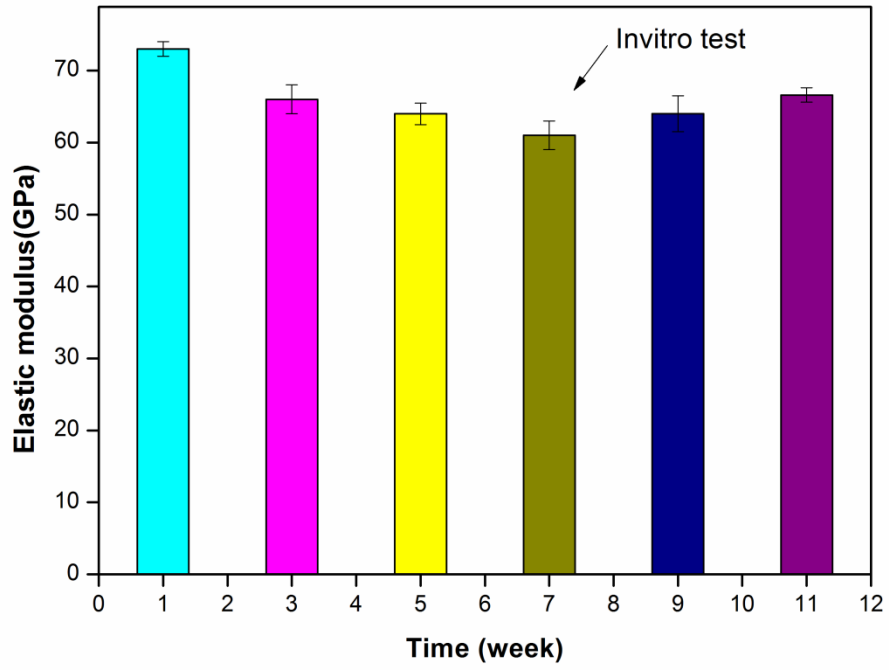
Sample	Density (ρ) (gm/cm^3)	V_L(m/s)	V_T(m/s)	Young's modulus E(GPa)	Shear modulus G(GPa)	Bulk modulus K(GPa)	Poisson's ratio (v)
BC1	2.45	4412	2315	34	13	29	0.3103
BC2	2.44	4552	2516	39	15	30	0.2801
BC3	2.42	4782	2729	44	18	32	0.2721
BC4	2.40	4932	2911	50	20	34	0.2326

3.3.5.1 Degradation of compressive strength of biocomposite during in vitro test

In vitro, the compressive strength and elastic modulus of the scaffolds after immersion in SBF in vitro are shown in Fig.3.6 as a function of immersion time. The strength and modulus decreased rapidly during the first three weeks, but later on, its decrease becomes slow. This trend was independent of in-vitro treatment. During the in-vitro test, the strength decreases from the fabricated value of 82±5MPa to 71±5MPa after seven weeks of treatment in SBF. After 11 weeks, the strength of the scaffolds immersed in SBF was 73±8MPa. The elastic modulus decreased from the fabricated value of 73±5MPa to 61±5MPa after seven weeks in SBF in vitro test. After 11 weeks, the strength of the scaffolds immersed in SBF was 66±8MPa.



(a)



(b)

Figure 3.6 Compressive strength (a) and Elastic modulus (b) as a function of time for bioglass biocomposites after immersion in simulated body fluid (SBF) in vitro test.

3.3.5.2 Elastic properties of Bioglass and HA reinforcement of biocomposites

In Fig.3.7 shows compressive strength and hardness value. Compressive strength and hardness values show between (30MPa to 99MPa) and (105MPa to 374MPa) in Fig. 3.7. The results indicate that with an initial addition of HA, the elastic modulus shows an unusual behavior. It increases with the further addition of HA content as shown in Fig. 3.8. In BC1, BC4 biocomposite, the measured young's, shear moduli range from 36 to 50 GPa and 12 to 20GPa respectively. Similarly, the young's and bulk moduli range from 36 to 50 GPa and 29 to34 GPa in Fig. 3.8 and Fig. 3.9 for BC1, BC4 biocomposites. The elastic modulus increases with an increase in the rigidity of bioglasses and HA content [A.V. Gayathri Devi et al. 2006].

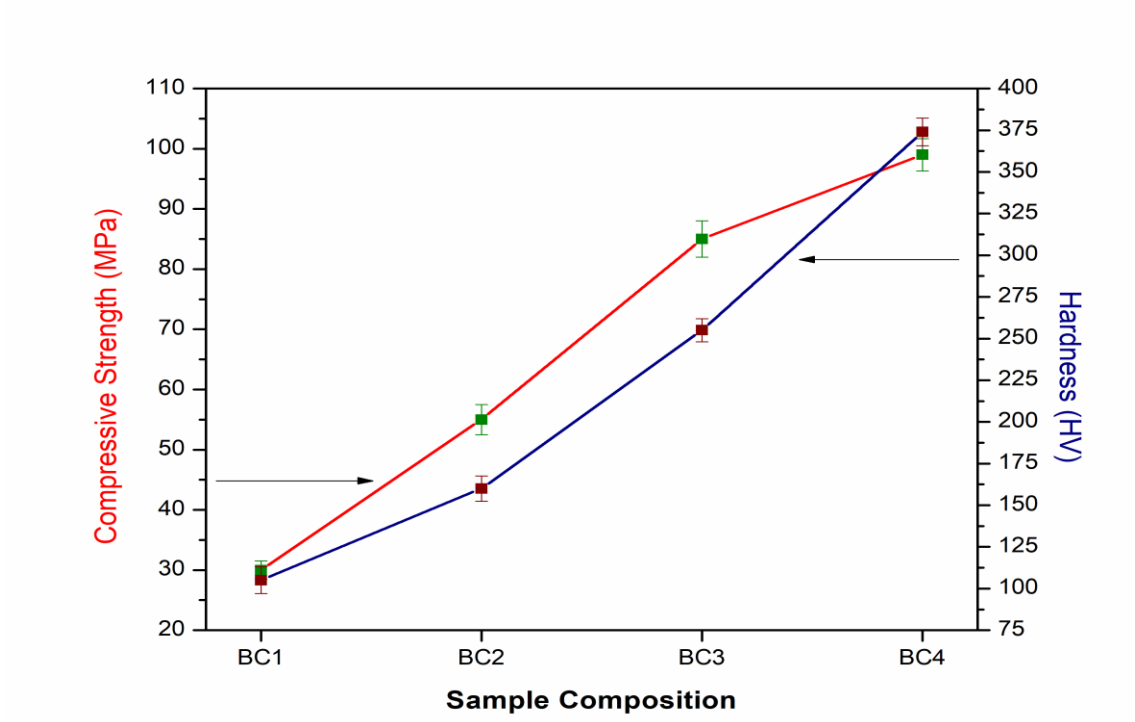


Figure 3.7 Compressive strength and hardness of biocomposites (BC1, BC2, BC3, BC4).

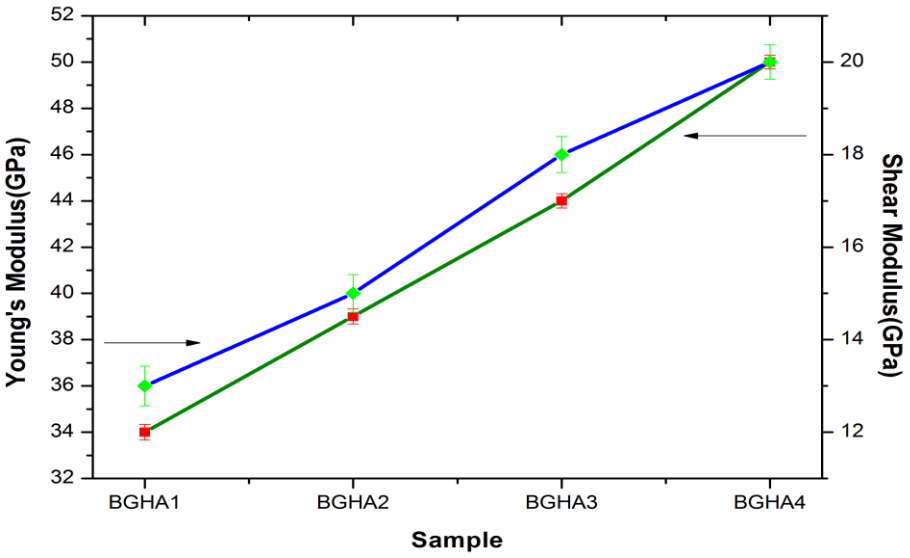


Figure 3.8 Young's modulus and shear modulus of biocomposites (BC1, BC2, BC3, BC4).

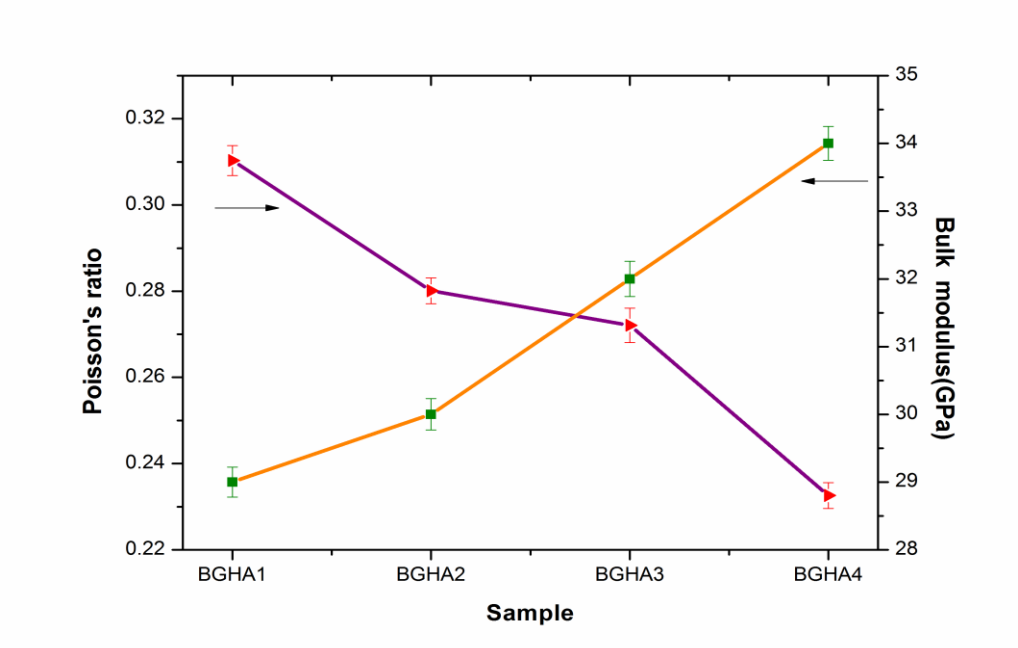


Figure 3.9. Bulk modulus and poisson's ratio of biocomposites (BC1, BC2, BC3, BC4).

3.4. Conclusions

The sintering process is used to prepare biocomposites with the addition of HA in bioactive glass (45S5). The thermal treatment of silicate-based glasses results in the release of stresses from the glass. There is a possible formation of crystalline phases along with the residual glassy phases. The increase of HA content in bioglass composites increase density, compressive strength, youngs, shear and bulk modulus while the Poisson's ratio remains nearly constant. Mechanical properties of the samples can be measured without any effect to the biocomposites. Mechanical properties show an excellent strength of biocomposites.

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