Chapter 5

# EFFECT OF SAMARIUM SUBSTITUTION ON BIOACTIVITY AND MECHANICAL PROPERTIES OF 45S5 BIOACTIVE GLASS

## 5.1 Introduction

A bioactive material is defined as a material that stimulates a biological response from the body, mainly bonding to soft as well as hard tissue. The name "Bio-ceramics" is a typical term used to cover glass ceramics and they are utilized as implant materials like bone. The main discovery of the bio-glass composition of (mol %) 46.1 $SiO_2$, 24.4 $Na_2O$, 26.9 CaO and 2.6 $P_2O_5$, and weight percent composition of 45% $SiO_2$, network modifiers of 24.5% $Na_2O$ and 24.5% CaO and 6% $P_2O_5$ to make for the glass composition [L. L. Hench et al. 1971]. This first generation biomaterials move toward the replacement of tissues was irreversibly altered when a special composition of soda-lime-phosphate-silicate glass was made by implanting in the femurs of rats in 1969 by Hench and colleagues at the University of Florida [C. A. Beckham et al.1971; T. K. Greenlee Jr et al. 1972]. The key shortcoming of 45S5 bioactive glass is connected tissue to its slow degradation rate. According to some past results, the mechanical properties of 45S5 bio-active glass were not wholly adequate for significant load bearing application [A. K Srivastava et al. 2012].

Bio-active glasses attach to the living bone through hydroxycarbonate apatite (HCA) layer formed on their surfaces after being dipped into a simulated body fluid (SBF) [M. Bohner et al. 2009]. The bio-active material should possess excellent biochemical behavior and biomechanical strength. The 45S5® bioactive

glass has a very good capability to bond with both soft and hard tissues [L. L. Hench et al. 1993]. Hench decided to make a glass of the $SiO_2$–$Na_2O$–CaO–$P_2O_5$ system containing high calcium contents with a composition close to a ternary eutectic in the $Na_2O$–CaO–$SiO_2$ diagram [L. L. Hench et al. 1971]. Substitution of bioactive glasses with different transition and rare earth metals such as Cerium, Gadolinium, Lanthanum, Samarium, Zinc, Manganese, Iron, Magnesium and Silver to change their biological and bioactive response has been studied by many research groups [J.R. Jones et al. 2006; M. Diba et al. 2012; Aylin M. Deliormanlı et al. 2015]. $Sm^{3+}$ ions play the important role as network modifier in $Sm_2O_3$-$SiO_2$–$Na_2O$–CaO–$P_2O_5$ glass system, as $Sm^{3+}$ possesses strong fluorescence intensity, high binding energy levels (135.6 BE/eV) and high quantum efficiency which further enhances optical as well as mechanical properties of glasses [J.L. Rygel et al. 2009; C. Leonelli et al. 2003; F.H. El Batal et al. 2008]**.** A biomaterial is a synthetic material to be used in intimate contact with living tissue. In present investigation, we study the effect of $Sm_2O_3$ substitution effect on the thermal, optical, mechanical and in vitro properties of the bioactive glass. $Sm^{3+}$ ions have been used in dental ceramics to mimic the fluorescence of natural teeth [V.K Vyas et al. 2016]. Samarium is also known to possess bacteriostatic properties and has low toxicity [Arepalli, Sampath Kumar et al. 2015]. 45S5 bioactive glass is a very successful biomaterial for clinical applications and many researchers have studied an incorporation of some ions such as Co, Ni, Li, Ti, K, Zr, Mg, and Sr in the base bioactive glass because of their unique outcome on osteoblastic cell proliferation of different ions in the base bioactive glasses [S.M. Salman et al. 2012; I. Kansal et al. 2011]. The present work is concerned with the preparation and characterization of Samarium containingBioactive Glass (BG). These bioactive

glasses were immersed insimulated body fluid (SBF) to validate the formation of a bone-like apatite layer on their surfaces by using FTIR. Surface morphology was studied using Scanning electron microscopy (SEM), and mechanical properties were studied in the present investigation.

## 5.2 Materials and methods

### 5.2.1 Preparation of bioactive glass

Samarium substituted 45S5 bioactive glasses with general weight composition (45-X) $SiO_2$. $XSm_2O_3$. 24.5 $Na_2O$. 24.5 CaO and 6 $P_2O_5$ where X= 0,1,2,3 and 4 wt% of $Sm_2O_3$ were prepared and presented in Table 5.1. Fine-grained quartz (99.9%) was used as a source of $SiO_2$. Analytical reagent grades $CaCO_3$, $Na_2CO_3$ and $(NH_4)H_2PO_4$, were used as a source of CaO, $Na_2O$, and $P_2O_5$, respectively. The required amounts of analytical reagent grade $Sm_2O_3$ were substituted in the batch as given in Table 5.1, for the partial substitution of $SiO_2$.

**Table 5.1:** ***Batch composition for $SiO_2$–$Na_2O$–CaO– $P_2O_5$–$Sm_2O_3$bioglass.***

| **Sample Id** | **Wt%** | | | | |
|---|---|---|---|---|---|
| | **$SiO_2$** | **$Na_2O$** | **CaO** | **$P_2O_5$** | **$Sm_2O_3$** |
| **BG** | 45 | 24.5 | 24.5 | 6 | 0.0 |
| **Sm1** | 44 | 24.5 | 24.5 | 6 | 1.0 |
| **Sm2** | 43 | 24.5 | 24.5 | 6 | 2.0 |
| **Sm3** | 42 | 24.5 | 24.5 | 6 | 3.0 |
| **Sm4** | 41 | 24.5 | 24.5 | 6 | 4.0 |

The raw materials for different samples were properly weighed. Then the mixing of different batches was done for 30 min in a ball mill of 480 rpm. After that, they were kept in a 100 ml alumina crucible (99.9%) and melted in globar furnace at 1400±10 ºC in the air as furnace atmosphere. The temperature of the furnace was

controlled within ±5 ºC by an automatic temperature indicator controller. The temperature of a furnace was held for 3 hours at 1400 ºC. After that, melted samples were poured onto a preheated aluminum sheet and directly transferred to a regulated muffle furnace at 520 ºC for annealing. After 2 hours of annealing furnace was switched off and glass was taken out at room temperature. Some part of the glass was powdered using an agate pestle and mortar for DTA, XRD, FTIR and SEM analysis, and the rest was cut into square shapes for determining mechanical properties.

### 5.2.2 Characterization

The characterization for samarium oxide substituted bioactive glass have been discussed in chapter 2 like Preparation of SBF, DTA, Powder X-ray diffraction (XRD) measurements, Structural analysis of bioactive glass by FTIR spectrometry, In vitro bioactivity study of bioactive glass, Mechanical Behavior Measurements, pH measurement, Surface morphology of bioactive glass sample by SEM, Elastic Properties of glass and cell culture of bioactive glasses using mouse fibroblast L929 cells lines.

## 5.3 Results and discussion

### 5.3.1 Differential thermal analysis (DTA) of $Sm_2O_3$ substituted bioactive glass

The thermal behavior curves of the bioactive glasses are shown in Figure 5.1, and the results dictate that the incorporation of $Sm^{3+}$ ions in the 45S5 bioactive glass decreased both the nucleation temperature from 601 to 598 °C and the crystallization temperature from 765 to 632 °C. Further, it was observed that the greater amount of modifiers in the composition has facilitated in lowering $T_g$ point and the viscosity of the glass melt [C.Y. Kim et al. 1989]. On substitution of

$Sm_2O_3$ for $SiO_2$ content in the composition caused the shift of exothermic peaks to lower temperatures as compared to that of the base glass. Therefore, lower energy is required to promote crystallization in the glass. This may be attributed due to the presence of larger cations in the system which increased interference in glass network. The modifiers occupied the interstitial position in the glass structure which decreased the oxygen bond strength [H. Tripathi et al. 2016]. Sampath et al. also found that an increase in the concentration of rare earth ions in the glassy system has allowed early nucleation by the nucleating agents like $P_2O_5$ in the glass composition. [A. S Kumar et al. 2015].

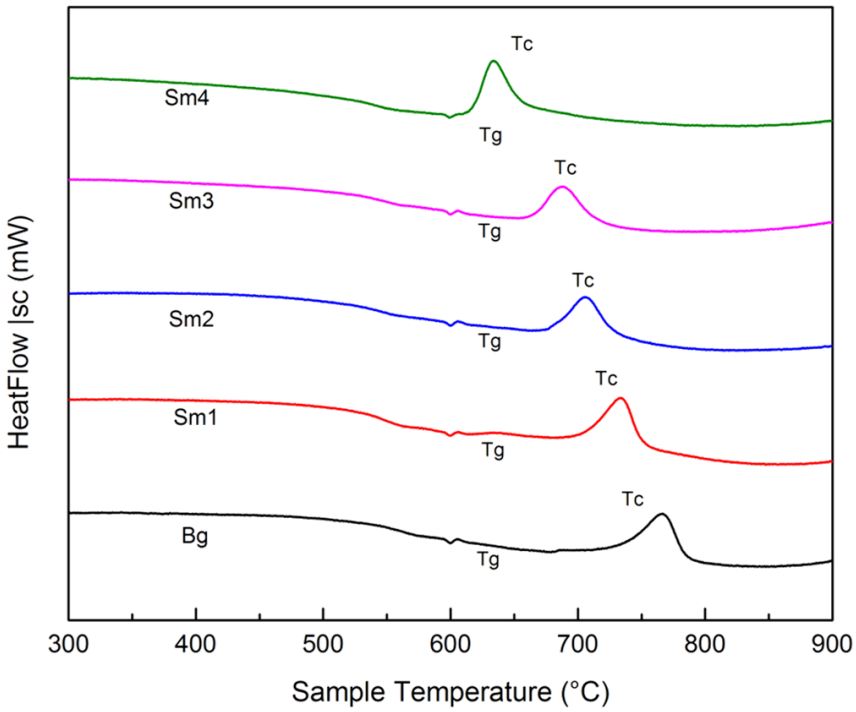


**Figure 5.1:** DTA image of$Sm_2O_3$ substituted bioactive glass.

### 5.3.2 XRD analysis and FTIR assessment

X-ray diffraction pattern of glass samples were presented from the Figure 5.2. It was observed there were no peaks, which indicates that bio-glass sample was amorphous in nature.

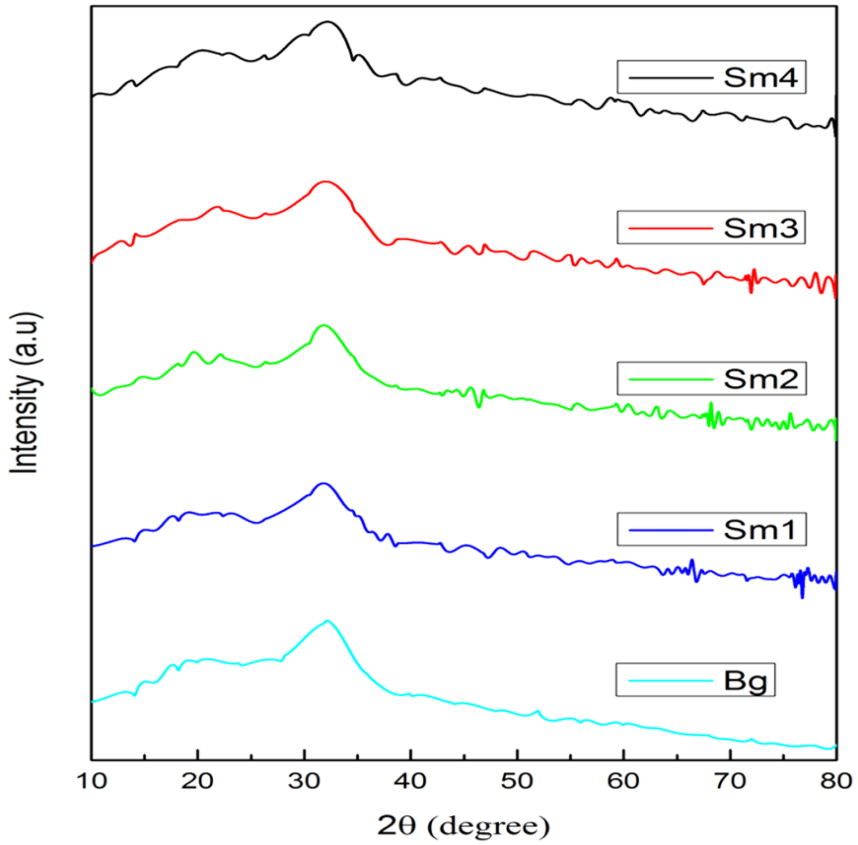


**Figure 5.2:** XRD image of$Sm_2O_3$ substituted bioactive glass

The Fourier transform infrared transmittance spectra of the bioactive glass samples were recorded in the frequency range of 400–4000 $cm^{-1}$ and were shown in Figure 5.3. The vibrational bands have been marked by the vertical dotted lines which were prepared similarly to transmission mode spectra, as given by earlier workers [E. Verne et al 2005]. The parent bioactive glass revealed the sharp bands successively at about 497, 1397, 2175, and 3790 $cm^{-1}$ indicating various functional group. The transmittance spectral bands of bioactive glasses were confirmed the main characteristic of the $SiO_4$ tetrahedral of silicate network. This was attributed due to the presence of $SiO_2$ as a significant constituent in the bioactive glasses.The resultant FTIR spectra at about 450 $cm^{-1}$ are associated with a Si–O–Si symmetric bending mode and the band at 775 $cm^{-1}$ correspond to Si–O–Si symmetric stretching of non-bridging oxygen atoms between $SiO_4$

tetrahedra. The major broadband at about 1397 $cm^{-1}$ was attributed due to Si–O–Si asymmetric stretching. The minor sharp peak at 1630 $cm^{-1}$ is associated due to stretching mode of C–O vibration of $CO_3^{2-}$ groups. The infrared frequencies and related functional groups were also reported in their bioglass® ceramic systems [F.H. El Batal et al. 2008]. Hydroxyl ($OH^-$) group peaks were found at 3506 $cm^{-1}$ as shown in Figure 5.3.

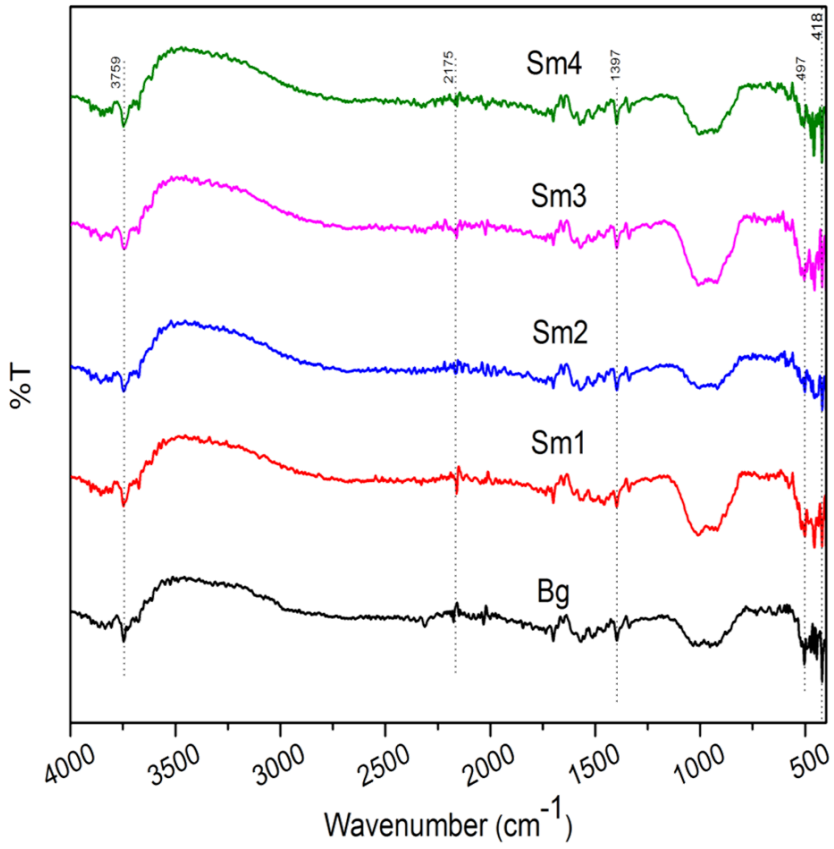


**Figure 5.3:** FTIR image of $Sm_2O_3$ substituted bioactive glass before SBF.

### 5.3.3 pH behavior in SBF

The variation of pH of bioactive glass samples after immersing in SBF solution up to 21 days were presented in Figure 5.4. It was observed that for all bioactive glass samples, the pH increase within 1–4 days as compared to the initial pH of the SBF solution at 7.4 under normal temperature and pressure condition. The increase in pH values is due to the fast release of cations through exchange with

$H^+$ or $H_3O^+$ ions in the SBF solution. The $H^+$ ions were replaced by cations which caused an increase in hydroxyl concentration of the solution [M.A.K. Elfayoumietal. 2010]. This leads to attack on the silica glass network, which results in silanols formation and this is due to decrease in pH after 3 days as indicated in Figure 5.4. When bioactive glass samples were immersed in SBF solution up to 13 days [Y. Gandhi et al. 2010], the maximum pH value is 9.93 of base bioactive glass. This is due to attack of $OH^-$ ions on the silica glass network, which results in silanols formation leading to decrease in pH as shown in Figure 5.4. It was also seen that the decrease in the pH of the SBF solution after 13 days was due to the breaking of glass network. The addition of $Sm_2O_3$ up to 4 wt%, increases pH of the SBF solution containing immersed samples which attend maxima after around 13 days and then it decreased with time referring to base glass sample. This dictates that addition of $Sm_2O_3$ up to 3.0 wt% in the glass samples increase its bioactivity, but after 3 wt% of $Sm_2O_3$ retards the bioactivity of the glass samples. Morphological properties of bioactive glasses also indicate that soaking in SBF solution leads to the formation of hydroxyapatite layer on the surface of the samples. The maxima of pH values were recorded between 1-8 days at pH 9.93, 9.88, 9.79, 9.61 and 9.37 for the samples BG–Sm4 respectively at 37 ºC under physiological condition, which is due to the fast dissolution rate. So, an increase in the pH value of SBF solution also favors the hydroxycarbonate apatite (HCA) formation. When the bone was formed, the cross-linking of the collagen chains and the subsequent precipitation of hydroxycarbonate apatite (HCA) is pH dependent and require a high pH at the bone formation site.

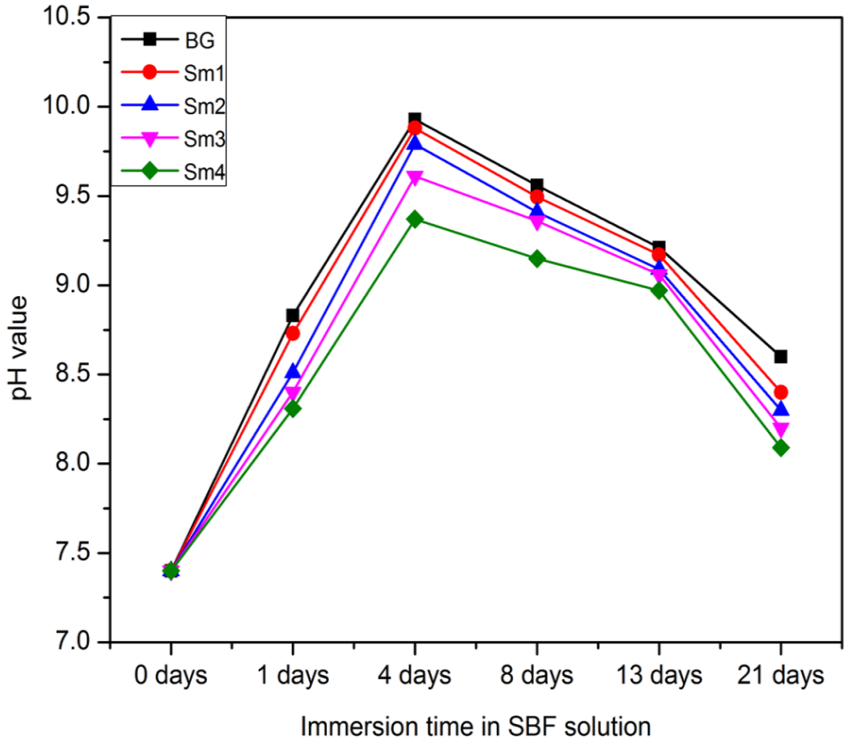


**Figure 5.4:** pH image of $Sm_2O_3$ substituted bioactive glass.

### 5.3.4 Surface morphology of bioactive glass samples by SEM

The surface morphology of bioactive glasses was analyzed before immersion in SBF solution by Scanning Electron Microscopy (SEM) and results are prepared in Figure 5.5. The samples were gold coated before SEM analysis. An SEM (Inspect 50 FEI) was used for determining the surface microstructure of bioactive glass samples. When the bioglass® sample was immersed in the SBF solution for different time periods, then Hydroxycarbonate apatite (HCA) layer was formed on the surface of bioactive glass samples. The SEM of the bioglass® surfaces pre-immersion reveals the form of many rod shapes structure and irregular grains after addition of $Sm_2O_3$ as shown in Figure 5.5, which are quite similar to the results observed. [Hanan et al. 2009]. Figure 5.6 shows the SEM micrographs of bioactive glass samples after soaking in SBF solution for 21 days. From the results it was found that bioactive glass samples which were soaked in SBF

solution for 21 days were covered with the irregular shape of HCA particles have grown into agglomerates. With SBF treatment HCA clusters change in a finer structure after 21 days of soaking due to partial dissolution and re-precipitation phenomena in solution. The bioglass® releases $Ca^{2+}$ and $Na^{+}$ ions from its surface via an exchange with the $H_3O^{+}$ ion in the SBF to form Si–OH or Sm–OH groups on their surfaces [Y. Gandhi et al. 2010]. This happens due to solution refreshing and signifying the formation of a continuous layer of HCA. After comparing these morphologies, it was concluded that the micrographs show the formation of HCA on the surface of bioactive glass samples after immersion in SBF solution for 21 days. EDX analysis also confirmed the formation of HCA layer after immersing the samples into SBF solution after 21 days as shown in Figure 5.6. Sampath at al. also investigated the substitution of barium up to 1.6 mol% in the base bioactive glasses. In his work, the SEM image showed the formation of HCA layer on the surface of the barium-containing bioactive glasses after putting in the SBF [T. Kokubo et al. 2006]. In a single investigation Samarium substituted glass enhanced hydroxyapatite formation and osteoblastic performance and antibacterial properties for bone tissue regeneration and also show the Sm substitute bioglasses exhibited improved osteoblastic cell response. The author observed that Sm-substituted 45S5 bioactive glass enhances the bioactive properties by the In-vitro studies.

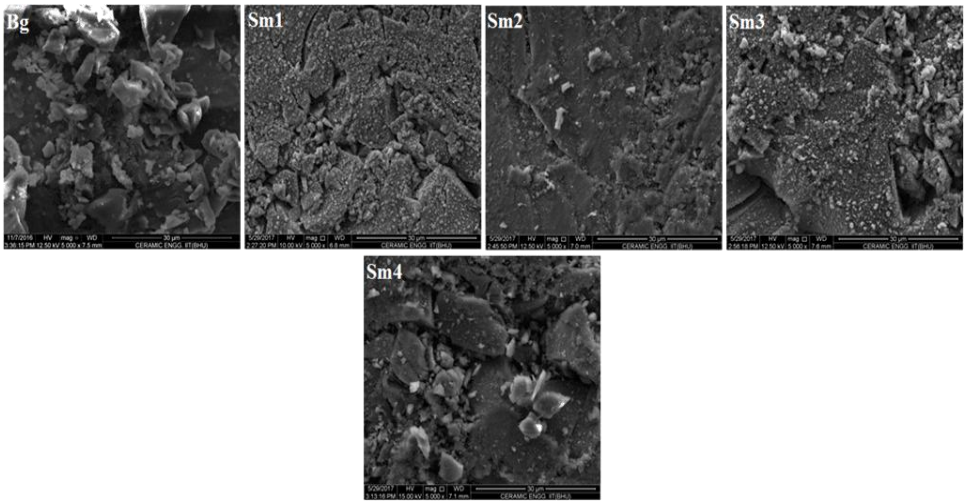


**Figure 5.5:** Morphological image (SEM) Samarium substituted bioactive glass before SBF treatment.

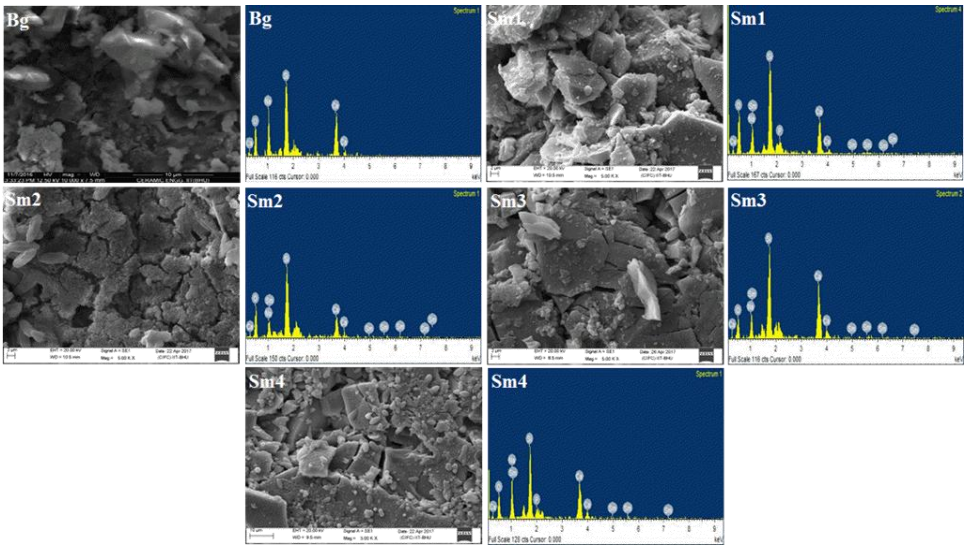


**Figure 5.6:** Morphological image (SEM) Samarium oxide substituted bioactive glass after SBF treatment for 21 days.

### 5.3.5 Weight loss measurement

Figure 5.7 shows the wt % losses of bioactive glasses, the un-substituted 45S5 glass showing less weight loss after soaking into SBF solutions. In all cases up to 20 days the weight loss is increasing proportionally upto 1.54% with time and after that weight loss is nearly constant for each sample. From the pH graph, the inference is already taken out that the pH is increasing due to the release of $Ca^{2+}$ and $Na^{+}$ ion into SBF solution that means weight loss in the Sm4 glass is more

which is suitably mention by the weight loss graph. It was establish that up to 4 days the weight loss in BG-Sm4 glasses is about similar trained. That means the glasses with higher content of Samarium possess the higher rate of dissolution, so the % weight loss is more.

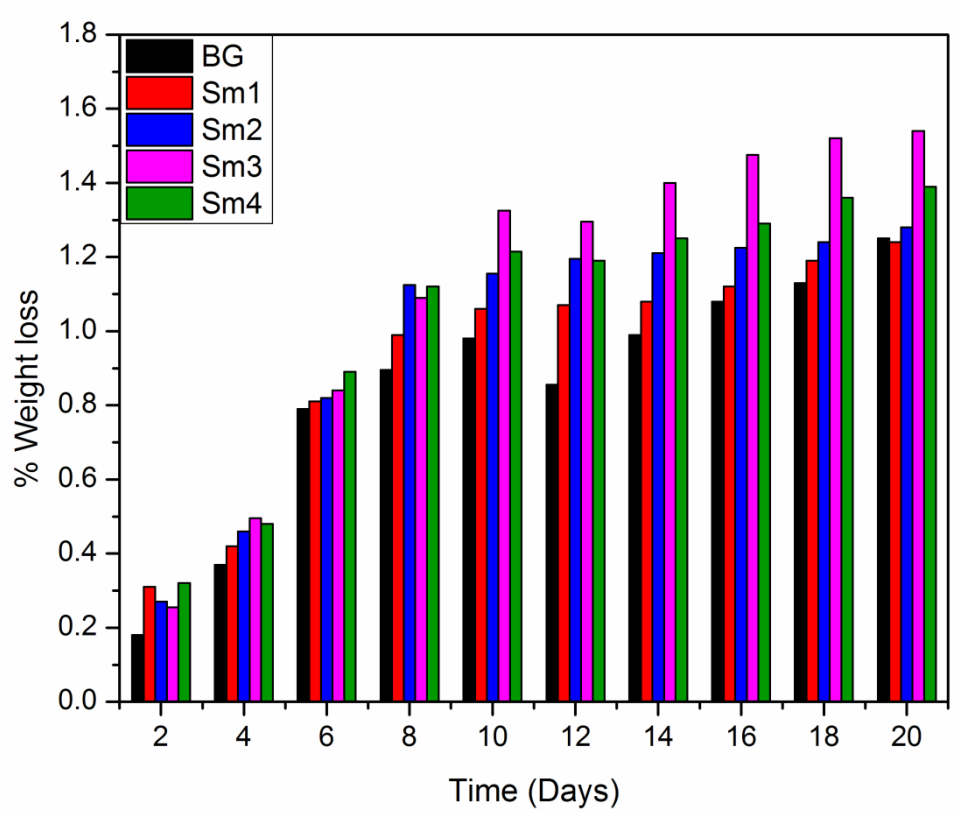


**Figure 5.7:** Weight loss measurement Samarium substituted bioactive glass upto 20 days.

### 5.3.6 Density and Mechanical behavior of base and $Sm_2O_3$ substituted glass

The density of the glass samples as a function of $Sm^{3+}$ within error bars were shown in Figure 3.8. It was found that an increase in wt% substitution of $SiO_2$ by $Sm_2O_3$ in a base glass, the density of glass samples increases from 2.69 to 2.71 g/cc respectively, this is due to small amount replacement of $SiO_2$ with $Sm_2O_3$ and is attributed due to the replacement of a light element (density of $SiO_2$ -2.65) with a heavier one ($Sm_2O_3$ -8.35).This is attributed due to the reason that the Samarium ions have occupied interstitial sites within the glass network [V.K Vyas et al. 2015]. Therefore, it increased the densities and resulted in creating new bonds

with the incorporation of $Sm^{3+}$ ions in the bioactive glasses. It caused reinforcement of glass structure and resulted in improvement in the compression of the glass samples.

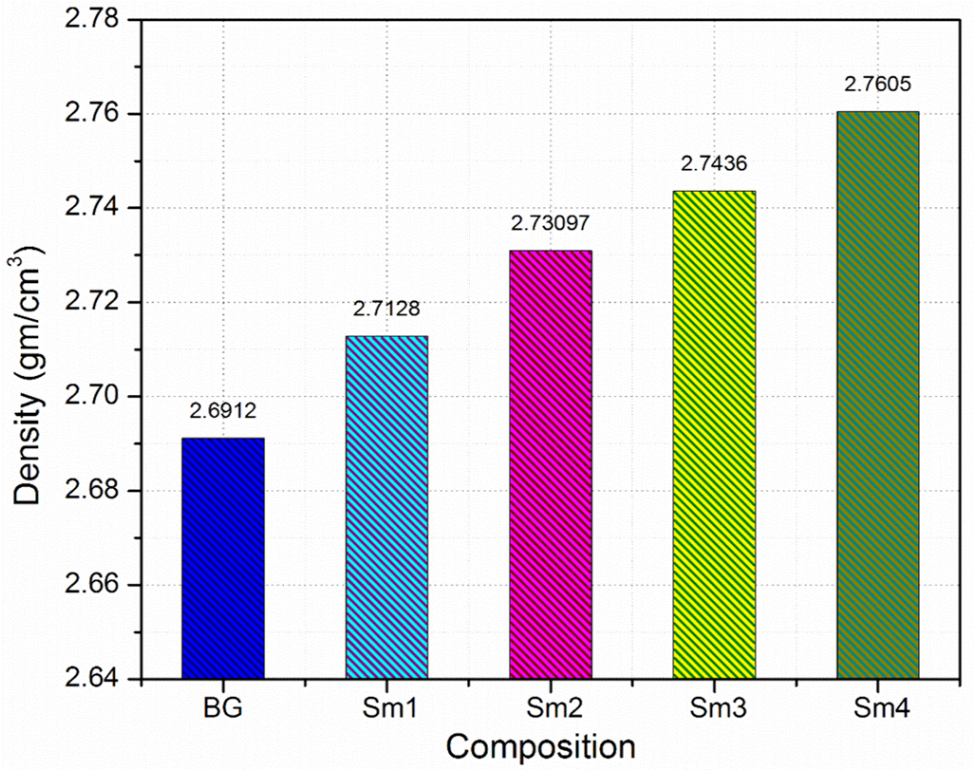


**Figure 5.8:** Density of Samarium oxide substituted bioactive glass.

### 5.3.7 Elastic modulus of $Sm_2O_3$ substituted bioactive glass

Figure 5.9, were represented the experimental values of elastic moduli, Young's modulus (E), shear modulus (S) and bulk modulus (K) of the bioactive glass samples and the value of longitudinal and share velocity of $Sm_2O_3$ substituted bioactive glass are shown in Table 5.2.

**Table 5.2:** ***Values of longitudinal and shear ultrasonic wave velocities.***

| **Glasses Sample** | **$Sm_2O_3$ substituted bioactive glass** | |
|---|---|---|
| | **$V_L$(m/s)** | **$V_T$ (m/s)** |
| BG | 5972 | 3459 |
| Sm1 | 6021 | 3481 |
| Sm2 | 6041 | 3497 |
| Sm3 | 6072 | 3524 |
| Sm4 | 6093 | 3551 |

The elastic moduli of the bioactive glass samples showed in Table 5.3 and found similar trends regarding improvement in their mechanical properties with the variation in the ultrasonic velocities. The elastic modulus of alkali silicate glasses was normally found to increase with increasing concentration of modifier above a critical limit as a result of an increase in cohesion [V.K. Vyas et al. 2015]. Thus, a greater bulk modulus of the glass samples was partially attributed due to the addition of more amounts of modifiers like $Na_2O$ and CaO in the silicate glass samples. The authors further mentioned that the effect of $P_2O_5$ on the elasticity of their glasses was not clear, although phosphorus was causing a more polymerized silicate network. Hench [L.L. Hench et al. 2010] has pointed out that Young's modulus of cortical bone is about 7–30 GPa, which is far below them most ceramic based implants. Hence, for biomechanical compatibility, the $SiO_2$–CaO–$P_2O_5$–$Na_2O$–$Sm_2O_3$ bioactive glass samples were found to be better. It was also known that the glasses and ceramics are brittle materials, as a consequence of which their handling and mechanical properties are not adequate for significant load-bearing applications. Therefore, a bioactive glass having the elastic modulus

higher than that of bone is required for clinical applications. So, there was an increase in Young's modulus from 76.363 to 78.894 GPa with increasing $Sm_2O_3$ content in the present base bioactive glass. Similar results were also obtained for shear and bulk moduli which showed improvement in the mechanical properties of bioactive glasses with varying ultrasonic velocities.

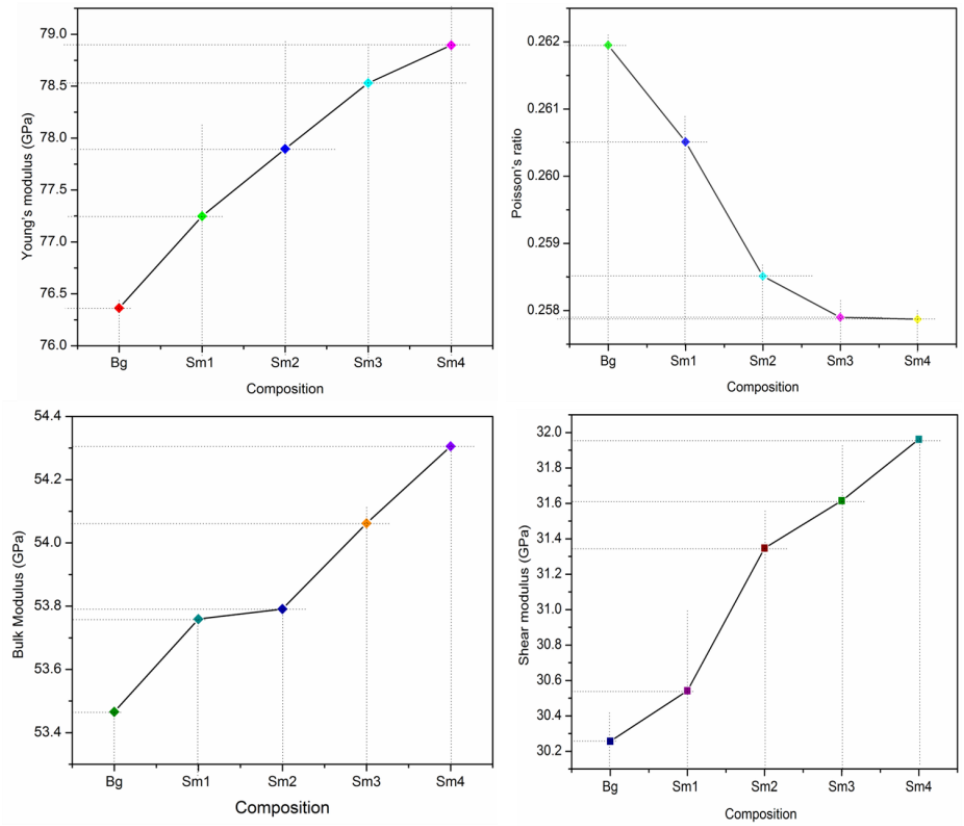


**Figure 5.9:** Young's modulus (E), shear modulus (S), bulk modulus (K) and Poisson's ratio of the $Sm_2O_3$ substituted bioactive glass samples.

## 5.4 Conclusion

In the present work, a comparative study was made on bioactive, physicochemical and mechanical properties of multi-component $SiO_2$-$Na_2O$-CaO-$P_2O_5$-$Sm_2O_3$bioactive glasses with varying concentration of $Sm_2O_3$. The following conclusions were drawn from these investigations. In the present investigation, it was found that Young's, shear and bulk modulus increase with increasing concentration of $Sm_2O_3$ in non-charge balance (NCB) glasses whereas the

Poisson's ratio decrease with increasing concentration of same oxides. After addition of $Sm_2O_3$, the nucleation and crystallization temperature decreases. In substitution to $Sm^{3+}$ ions, it was found that physical properties, as well as biological properties, increased with increasing concentration of $Sm_2O_3$. XRD pattern of modified 45S5 glass shows the amorphous phase behaviour in the presence of $Sm^{3+}$ ions. SEM and EDX image showed that HCA layer present on the surface of samples after immersion in SBF solutions. FTIR spectra showed the structural behaviour of base and $Sm_2O_3$ substituted bio-glass. Higher content of Samarium possess the higher rate of dissolution, so the % weight loss is more.