6.1 Introduction

The bioactive behavior of the biocomposite by an implant in a direct bone can improve the bonding between the implant and the surrounding bone tissue. Bioglass 45S5 was developed by Hench exhibits strong bioactivity [L.L. Hench et al. 2006]. First manufactured bioactive glass was the bioglass 45S5 also called as the Hench bioglass [L.L Hench et al. 1973]. A melt derived, quaternary bioglass constituting SiO_2, Na_2O, CaO and P_2O_5 in specific proportions. Hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$, HA) is a calcium phosphate with high biocompatibility and widely used in biomedicine to make bone and teeth implants. It is the main component of human bone [N. Roveri et al. 2010]. HA is a very suitable compound to be used as the sunscreen. It is nontoxic and already present in the human body. However, unmodified HA on its own does not absorb UV radiation. HA can be used as UV absorber if included in an appropriate matrix [D. Holzmann et al. 2009], or combined with other organic compounds, as ascorbic acid [R.M.Amin et al. 2016]. Doping HA with ions such as iron, manganese, and zinc can also make it a UV absorber [De. Araujo et al. 2010; T.S. De Araujo et al. 2010].

The bioactivity of a material is defined as the potential to form bone-like hydroxyapatite when transplanted into bone tissue. SBF is a non-cellular solution that emulates human blood plasma. So, SBF consists of ions in the same concentration range as human blood but does not contain proteins, hormones, glucose, and vitamins. It was initially developed by Kokubo to reproduce in-vitro apatite formation by bioactive materials such as bioactive glasses with distinct biomaterials. Thus, an initial prediction can be made about the bioactivity and the corrosion behavior of a novel biomaterial [S.B. Cho et al. 1995; C. Ohtsuki et al. 1995;C. Ohtsuki et al. 1991]. SBF

solutions cause structural and chemical changes on the surface of the material. In principle, three processes occur in parallel: leaching, degradation, and precipitation [M. Cerruti et al. 2005; L.L. Henchet al. 1993]. First, the leaching process is characterized by a release of alkaline metals and alkaline earth metals, especially by exchange of the cations H^+ and H_3O^+. The ion exchange increases the pH-value at the interface up to 7.4. Second, the degradation of the material occurs in parallel with the breakdown of silica-oxygen bondings by hydroxyl ions. This breaking down occurs nearby. Subsequently, silicon in the form of silicic acid $Si(OH)_4$, is released into the SBF solution. The degradation will decrease if the glass consists of more than 60 wt% of SiO_2 due to the respective high level of oxygen bridges within the glass structure. The hydrated silicic acid (SiOH) formed at the surface is surrounded by at least one hydroxy group bound to a silicon atom (silanol) by polymerization of neighboring organic silicon compounds. Thereby a silicic acid gel layer forms. In parallel, calcium and phosphorus are released from the glass by precipitation and form a calcium phosphate-rich phase on the surface. This calcium phosphate phase in the gel layer is initially amorphous (a-CaP) in nature. Subsequently, the CaP phase crystallizes into carbonated hydroxyapatite (HCA) structure by the carbonate anions from the solution[W. Cao et al. 1996]. The essential feature of 45S5 material includes the weight percentage of SiO_2 in the glass matrix, being 45%, in conjunction to its increased content of Na_2O, CaO and the appropriate corresponding value of the ratio CaO/P_2O_5 [F. Baino et al. 2011].

Bone-bonding of bioactive glass is the ability of the implant-glass to react chemically with the living tissue in the presence of body fluids by a number of successive chemical surface reactions. The final product of these reactions is a formation of carbonated hydroxyapatite layer. Hench and West proposed a complex process for the formation of

the apatite layer involving five main reaction steps including. Step 1: Dealkalization of the surface by exchange of cations (Na^+ or Ca^{+2}) with H^+ or H_3O^+. Step 2: Loss of soluble silica in the form of $Si(OH)_4$. Step 3: Repolymerization of $Si(OH)_4$ leading to condensation of a SiO_2 rich layer on the glass surface. Step 4: Migration of Ca^{+2} and PO_4^{-3} groups to the surface forming $CaO\text{-}PO_4^{-3}$ clusters on the top of the SiO_2-rich layer, followed by growth of the amorphous $CaP(CaO\text{-}P_2O_5)$ film. Step 5: Crystallization of the amorphous CaP by incorporation of OH^-, CO_3^{-2} anions from the solution to form a hydroxyl carbonate apatite layer ($Ca_5(PO_4)_3(OH)$, HCA) [LL. Hench et al. 1996; J.P. Zhong et al. 2000]. The opportunity for in vivo apatite formation was elucidated by using the simulated body fluid, including ion concentrations approximately equal to those of human blood plasma [T. Kokubo et al. 1990]. The in-vivo bone bioactivity of various types of materials can be predicted through the apatite formation on its surface in SBF and can be tested in vitro by the apatite formation in SBF.

Iron ions are expected to exist in Fe^{3+} and Fe^{2+} valence states in these glasses. Fe^{3+} ions participate in the glass networking in both tetrahedral and octahedral coordination, whereas Fe^{2+} participate in octahedral condition and act as modifiers [V. Vercamer al. 2015; G. Nagarjuna et al. 2009]. The simultaneous presence of iron ions in divalent and trivalent oxidation states with different coordination will have a strong behavior on the bioactivity of the base glass. Mixing of CaF_2 to the base glass normally lowers the viscosity of the glass melted and stimulates the growth of HA layer on base glass [G. Krishna et al. 2008]. New approaches for the preparation of magnetic bone substitutes have been described, like bioactive (Fe^{+2}/Fe^{+3}) doped hydroxyapatite (Fe-HA) with superparamagnetic like properties as well as hydroxyapatite-based scaffolds with

magnetic properties attained by dip-coating in aqueous ferrofluids containing iron oxide nanoparticles. The aim of attracting and taking up in vivo growth factors, stem cells or other bio-agents is to bound to magnetic particles via magnetic driving, but they are not intended for the hyperthermic treatment of bone tumors. The most common method to yield HA with magnetic properties is the fabrication of iron-substituted apatite [M. Jiang et al. 2002]. Another possible substitution may result in magnetic properties is the substitution of cobalt ions for calcium ions in the HA crystal lattice. There have been limited studies recently on the substitution of cobalt into the HA lattice. The synthesized CoHA via hydrothermal treatment where Co^{2+} substituted for Ca^{2+} up to about 12 % [Z. Stojanovic et al. 2009; L. Veselinovic et al. 2010].

6.2 Material and Methods

6.2.1 Preparation of biocomposites

Bioactive glass 45S5 powders with a chemical composition close to 45S5 Bioglasss® containing analytical grade (45% SiO_2, 24.5% Na_2O, 24.5 CaO and 6% P_2O_5) in weight percentage were prepared by melting at 1400-1410°C in a vertical muffle furnace. The melt was held for 4 hours for homogenization and poured into a graphite mold step by annealing at 450-500°C. HA was prepared by sol-gel route block diagram shown in Fig.2.3. To make the analytical grade Fe_2O_3 (2.5,5.0,7.5,10.0wt%) and Cobalt Oxide (1,1.5,2.0,2.5wt%) based composites, four different compositions were prepared at Ceramic Engineering, IIT(BHU), Varanasi, India). The composition of biocomposite sample were shown in Table 6.1. The homogenous mixture of metallic alloy and ceramic component was obtained by mixing in a planetary ball mill for 1 hour under an argon atmosphere to avoid oxidation. Mixed powders were uniaxially pressed under

800 MPa to prepare green pellets of composites. Samples were sintered by controlled heat treatment at 1000°C. Powdered samples were used for the characterization of XRD.

Table 6.1: Composition of Bio-composite (BGHAFeCo1, BGHAFeCo2, BGHAFeCo3, BGHAFeCo4).

Bio-composite Samples	Composition (wt %)			
	BG (45S5)	HA	Fe_2O_3	CoO
BGHAFeCo1	80	16.5	2.5	1.0
BGHAFeCo2	70	23.5	5.0	1.5
BGHAFeCo3	60	30.5	7.5	2.0
BGHAFeCo4	50	37.5	10	2.5

Processing methods for BGHAFeCo biocomposite play an important role in the properties of the Iron and cobalt oxide composites. Powder metallurgy has many advantages in the processing of glass matrix composites. It can be used to synthesize a wider range of compositions at low cost with characteristic isotropic properties [Y. Liet al. 2009; C. Wen et al. 2002]. Fig.2.4 shows the powder metallurgy process to fabricate BGHAFeCo biocomposite.

6.3 Result and discussion:

6.3.1 Differential thermal analysis (DTA) / Thermogravimetry Analysis (TGA)

DTA curves of biocomposite in Fig.6.1 show the nucleation temperature (endothermic peak) at 438˚C and crystallization temperature (exothermic peak) at 817˚C respectively. During heat treatment process, the intermediate oxides may switch their structural role into the glass based biocomposite. The network modifiers can (creating non-bridging oxygen and disrupting the silicate glass network) enter into the silicate network. The TGA is an efficient technique to evaluate the thermal stability of the bioglass based biocomosite. It can be seen from Fig.6.1 that the weights of bioglass based biocomposite has been increased with the increasing temperature. It may be due to formation of respective crystalline phases which was confirmed by XRD Fig.6.2. The thermal decomposition of the bioglass powders were measured upto 900˚C in air during the thermal treatment process. Thereby the developed polycrystalline phases have different molecular weights [L.L. Hench et al. 1998; O. Bretcanu et al. 1989; V.R. Mastelaro et al. 2000]. This is the source for increasing weight. One can monitor that the weight of samples has been increased after reaching their nucleation temperatures around 580˚C and the thermal decomposition also observed upto 230˚C which might be due to absorbed water.
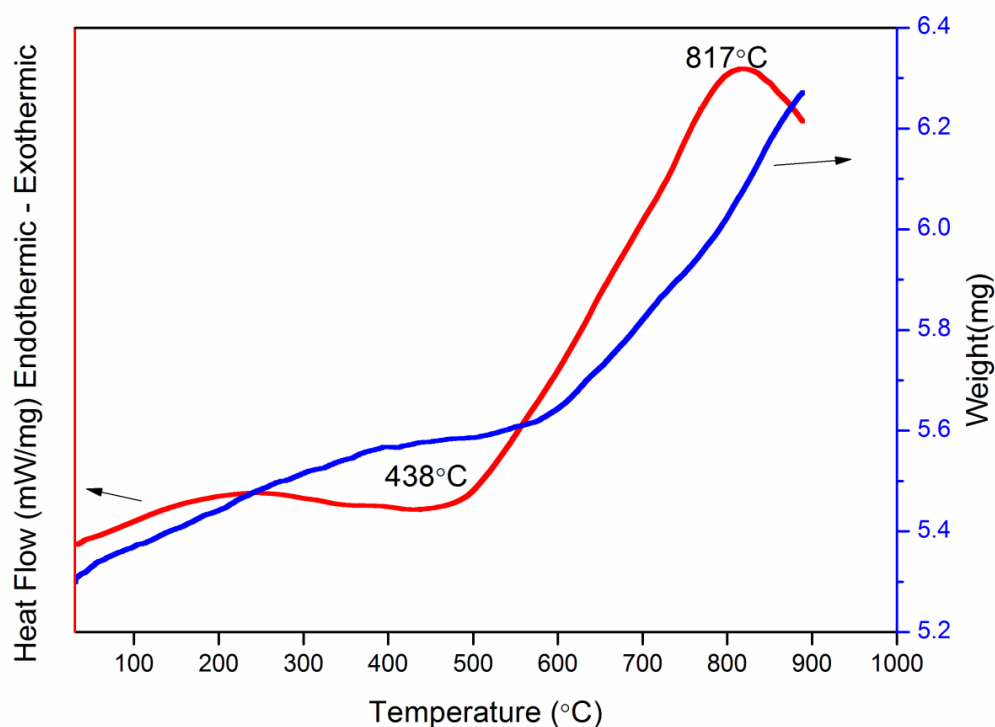
Figure 6.1 DTA/TGA of BGHAFeCo sample.

6.3.2 XRD analysis of biocomposites

The typical XRD patterns were obtained from the surfaces of biocomposites before and after soaking in SBF for various time periods (0, 1, 3, 7, 14 and 21 days). XRD patterns of the biocomposite sample with Fe2O3 (2.5,5.0,7.5,10.0 wt%) and CoO (1,1.5,2.0,2.5wt%) is shown in the Fig.6. After immersion in SBF, crystalline peaks appear in the XRD patterns, indicating the formation of a crystalline layer on the surface of the biocomposites. Initially, well-defined hydroxyapatite (HA) peaks develop at (2θ) values of 35-37° of these composites after three days of soaking in SBF. And Fe2O3 peaks develop at (2θ) values of 31-33° also cobalt peaks developed at (2θ) values of 44° after seven days of immersion. XRD pattern of BGHAFeCo was obtaining after immersed in SBF for 21 days. It shows from the figures that HA layer formation follows the same trend as the case of BGHAFeCo immersed in SBF. Closed

look at the XRD patterns of BGHAFeCo confirms a comparatively rapid growth of HA layer due to the presence of bone phases at its surface. The rapid increase in the crystallite size, especially at early hours of immersion on the surface of BGHAFeCo indicates the strong bioactive character of the biocomposite sample which is primarily due to the large amounts of bone mineral phase present in the sample. Hench first proposed a sequence of reactions occurring at the surface of silica-based bioactive glasses immersed in SBF, leading to the formation of a Ca–P rich layer on the surface of the glass [L.L. Hench et al. 1991]. The biocomposite samples are controlled heat-treated at 1000°C to obtain crystallized samples. The biocomposite samples produce magnetite, apatite ($Ca_5(PO_4)_3(OH)$) and wollastonite ($CaSiO_3$) phases as confirmed by XRD. The formation of $CaSiO_3$ phase is expected as the CaO tends to react with SiO_2 to produce this kind of phase. Magnetite (Fe_3O_4) phase is responsible for the magnetic properties in the biocomposite samples. It is formed in the biocomposite matrix by the reaction:

$$6Fe_2O_3 \quad \rightarrow \quad 4Fe_3O_4 \quad + \quad O_2 \quad \text{---------------------} (6.1)$$
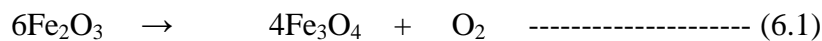
The conversion of hematite into magnetite depends on the heat treatment of the base glass. It is observed that the intensity of peaks corresponding to magnetite phase has increased with an increase in Fe_2O_3 content [K. Sharma et al. 2012].
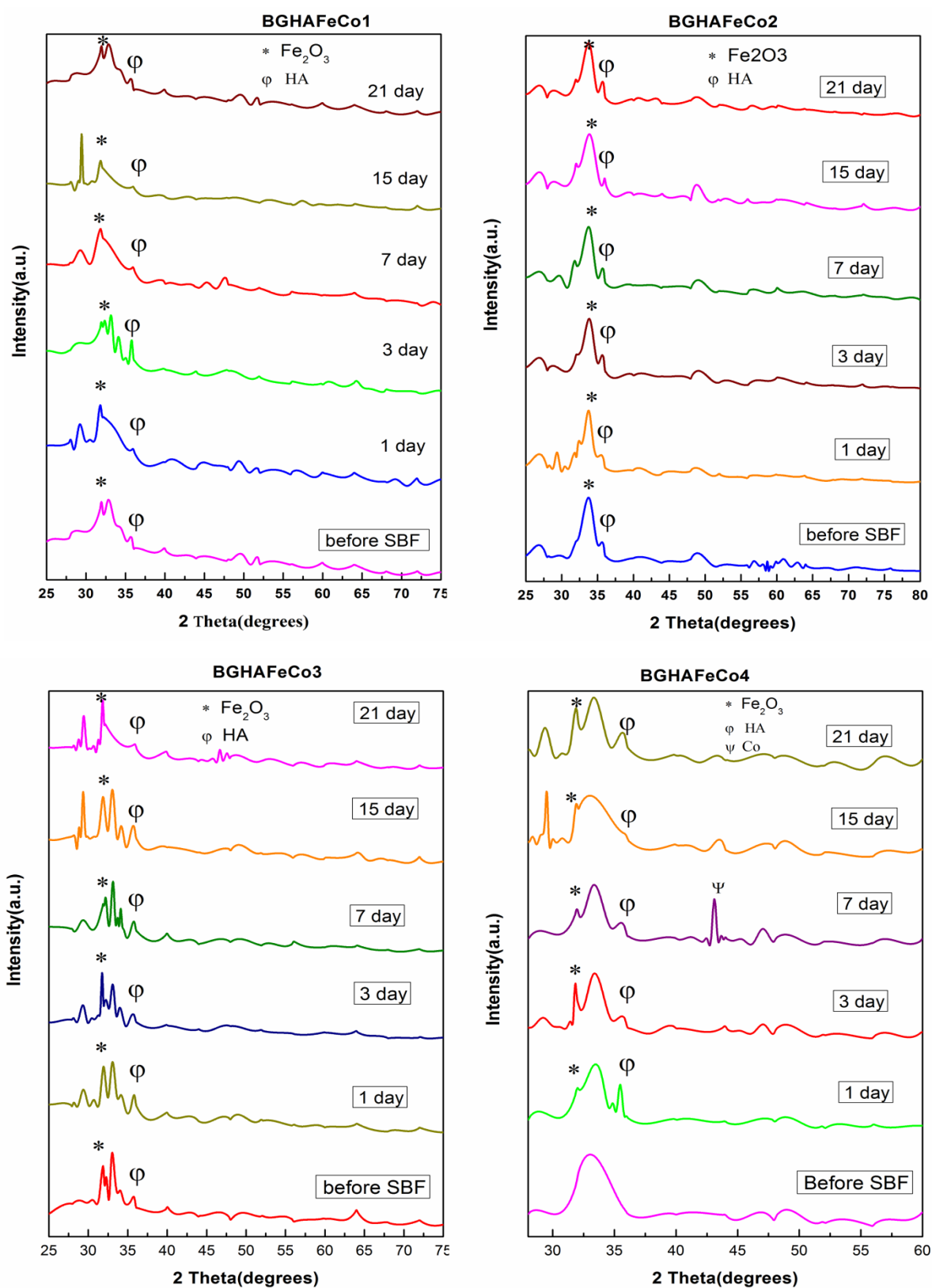
Figure 6.2 XRD pattern of biocomposite sample BGHAFeCo1, BGHAFeCo2, BGHAFeCo3 and BGHAFeCo4 before and after SBF treatment (1,3,7,14,21) days.

6.3.3 In vitro bioactivity of bioactive glasses by FTIR spectrometry

The FTIR transmittance spectral bands of the bioactive glasses were obtained before and after immersion in SBF for different time periods such as 1, 3, 7, 14, and 21 days. In general, it is well known that a decrease in the intensity of transmittance bands indicates an increase in the molecular concentration of species formed at the surface of the bioactive glass when treated with SBF solution with an increase in soaking time. The present results obtained by FTIR spectrometry were also in good agreement with the earlier studies made by previous workers [C.Y. Kim et al. 1989; M.R. Filgueiras et al. 1993; I. Rehman et al. 2000]. Fig. 5 BGHAFeCo1 sample shows the IR spectral bands before and after treatment with SBF. The new bands were revealed after one-day treatment with SBF at 488 and 572 cm^{-1} which are attributed to (phosphate) P–O bending. The bands at 1504 and 1697 cm^{-1} are assigned due to (carbonate) C–O stretching mode of vibration and broad band at about 3740 cm^{-1} was due to the presence of (hydroxyl) O–H groups on the surface. The prolonged period in SBF shows the similar behavior with a small decrease in the intensities of the bands which resulted from the formation of HCA layer on the sample surface. Fig. 6.3 BGHAFeCo2 sample shows the bands before and after immersion in SBF. The new bands appeared after immersion in SBF for one day at 448 and 569 cm^{-1} are related to (phosphate) P–O bending. The bands at 1472 and 1582 cm^{-1} correspond to (carbonate) C–O stretching mode and the broad bands at about 3740 cm^{-1} are assigned as usual due to the formation of (hydroxyl) O–H groups on the surface of the sample. The prolonged period of the treatment of sample in SBF shows the same behavior with a small decrease in the intensities of the bands which resulted in hydroxyl carbonated apatite layer formation on the sample.

Fig. 6.3 BGHAFeCo3 sample shows the bands before and after immersion in SBF. The new bands recorded after immersion in SBF for one day at 448 and 569 cm^{-1} and they are associated with (phosphate) P–O bending. The bands at 1494 and 1593 cm^{-1} correspond to (carbonate) C–O stretching mode and broad band at about 3729 cm^{-1} are attributed due to the presence of (hydroxyl) O–H groups on the surface of the biocomposite sample. The prolonged period of the sample in SBF shows the similar behavior with a small decrease in the intensities of the bands, and this dictated for the hydroxyl carbonate apatite layer formation. Fig. 6.3 BGHAFeCo4 sample shows the bands before and after soaked in SBF. The new bands were recorded after one day soaking in SBF at 492 and 580 cm^{-1} which are associated with (phosphate) P–O bending. The FTIR bands were obtained at 1395 and 1516 cm^{-1} correspond to (carbonate) C–O stretching mode of vibration and the broad bands at about 3729 cm^{-1} was attributed due to (hydroxyl) O–H groups on the surface of the sample. The prolonged period of the sample in SBF shows the similar behavior with a small decrease in the intensities of the bands which favored for the formation of hydroxyl carbonated apatite layer. It may be noted from FTIR spectra as given in Figs.6.3 show that the Fe_2O_3(2.5-10wt%) and CoO(1-2.5wt%) substitution in base bioactive glass at the cost of silica did not affect apatite formation.
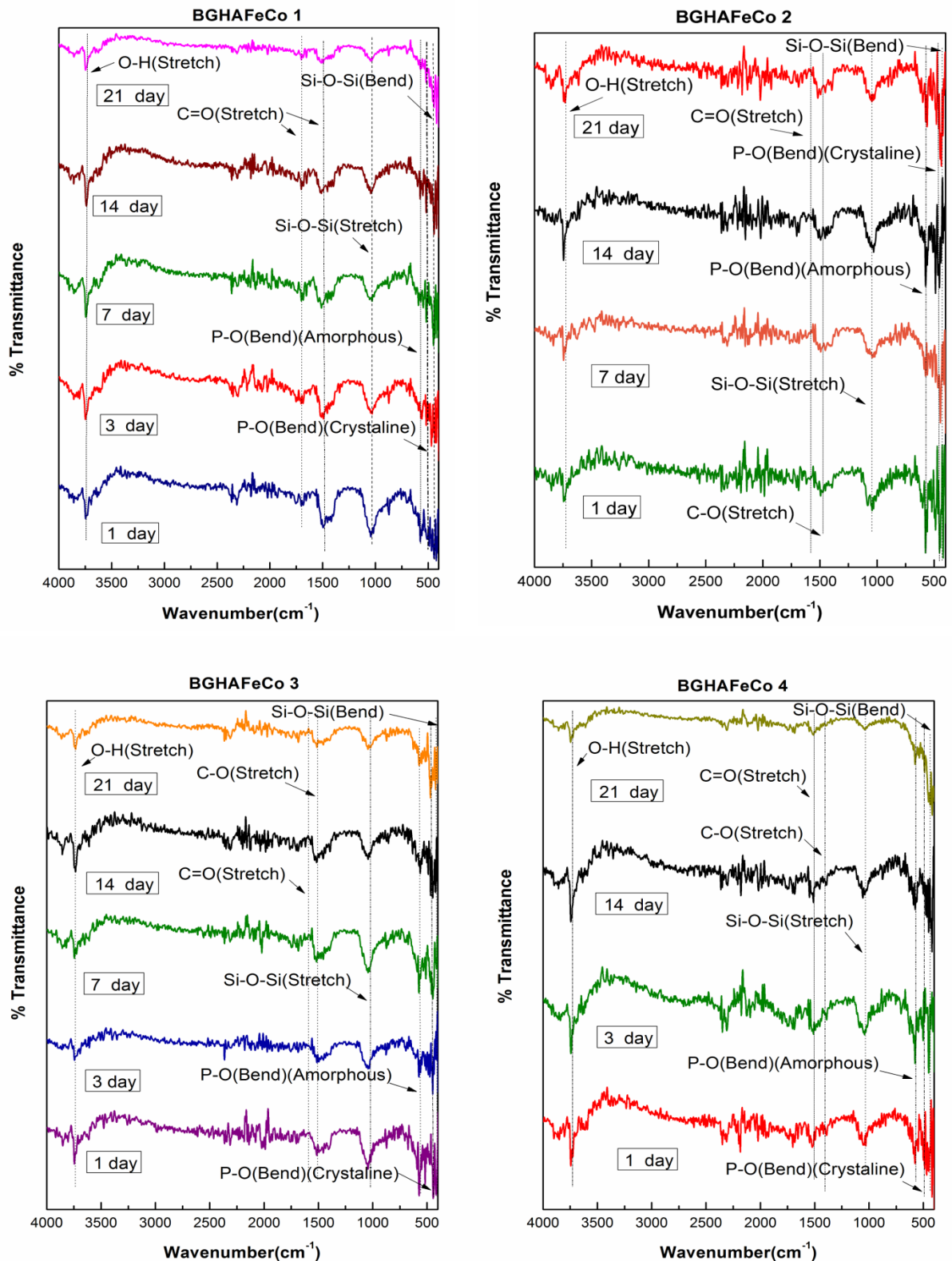
Figure 6.3FTIR of the biocomposite sample BGHAFeCo1, BGHAFeCo2, BGHAFeCo3

and BGHAFeCo4 before and after immersion in SBF treatment for (1,3,7,14,21) days.

6.3.4 Magnetic properties of biocomposites

Room temperature magnetization of BGHAFeCo4 as a function of the applied field is depicted in fig 6.4(a). This sample exhibit nonlinear variation of magnetization with field and the inset in the figure shows that the sample exhibit magnetic hysteresis with the coercive field (Hc) and finite magnetic retentivity (Mr). Fig.6.4(b) depicts the thermos-magnetic behavior of BGHAFeCo4 undergoes a two-step reduction to zero magnetization on the heating cycle. Sample depicts one step increase in magnetization on the cooling cycle. Inset in Fig. 6.4(b) depicts the linear variation of the inverse susceptibility χ-1 with temperature in the range of 950°K to 1050°K. Table 3 list the magnetic parameter of BGHAFeCo4. The magnetization experiments for BGHAFeCo4 sample is carried out in a magnetic property measurement system (MPMS3, QUANTUM Design, USA) at room temperature. DC magnetization is a function of temperature (M-T) during both zero field cooling (ZFC), and field cooling (FC) at 500 Oe was observed in Fig.6.4(b) The M-T curves exhibit a drop crossover around ~250 K. This may be expected that there is a ferroelectric transition (Tc) on this temperature as reported by Phokha et al. [S. Phokha et al. 2012]. It is found that observed ferromagnetism in our samples is due to spin canted of Fe^{+3} as the source of the magnetic moments. In BGHAFeCo(1-3) sample shows less magnetization but BGHAFeCo4 biocomposite results depict more magnetization. So, BGHAFeCo is not very good for bone implantation in a human body.

Table 6.2: Magnetic parameters and crystal size of BGHAFeCo4 samples.

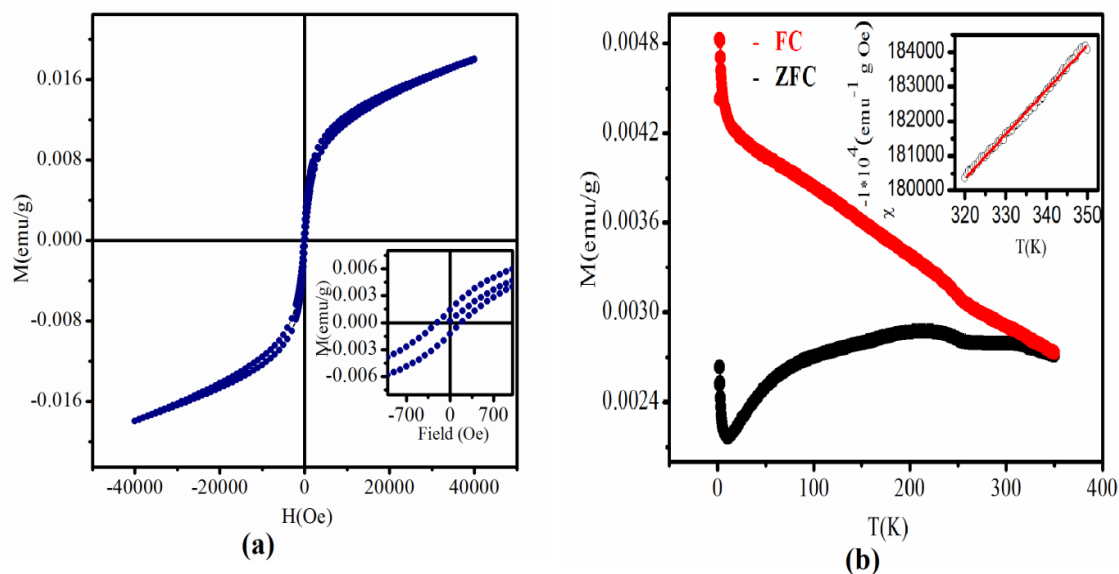| Sample	M_s(emu/g)	M_r(emu/g)	Coercivity (H_c)
BGHAFeCo	0.018	0.00138	191.775

(a) **(b)**

Figure 6.4(a) Room temperature M-H curve of BGHAFeCo4 sample. Inset shows an expanded view of data close to the origin. (b) M-T curves of BGHAFeCo4 recorded during heating and cooling cycles under an applied field of 1kOe. Inset shows the linear fits to χ^{-1}-T data of BGHAFeCo4 sample recorded above their T_C.

6.3.5 pH change during immersion

HA formation and growth on the surface of the biocomposite sample is a pH-sensitive reaction. Careful measuring of pH can help in understanding the mineralization activity of the sample. Mechanism of HA formation in bioactive silica-based composite materials can be described regarding pH variation with immersion in SBF. At the initial value of pH 7.40 of the SBF, the reaction mechanism starts through an ionic exchange

of H_3O^+ from the SBF by cations of glass such as Na^+, Ca^{+2}, etc. It induces the formation of an amorphous hydrogel silica layer resulting in a sudden increase in pH from 7.4 to 8.0 at the interface. This exchange leaves a silica hydrogel layer formed on the surface before the formation of the HA layer. At this high pH value, part of the silica hydrogel is dissolved in the SBF and precipitation of an HA layer takes place on the surface of the material. Several reports have confirmed that various intermediate calcium phosphate species precipitate at different pH intervals during ion exchange. Silanol group formation and silicon dissolution in SBF were higher for sol-gel derived 58S (58 SiO_2, 33 CaO, 9 P_2O_5wt%), 68S (68 SiO_2, 23 CaO, 9 P_2O_5wt.%), 77S (77 SiO_2, 14CaO, 9 P_2O_5 wt.%), 91S (91 SiO_2, 9 P_2O_5 wt.%) glasses than melt quenched glasses 45S5 and 60S (60 SiO_2, 17 CaO, 17 Na_2O, and 6 P_2O_5 wt.%) [D. Arcos et al. 2003]. The ion exchange mechanism is more rapid in the sol-gel driven glass as compared to melt quenched counterparts mainly because of the larger surface area of the sol-gel derived samples [N. Shankhwar et al. 2015].

$$HCO_3^{-3} \longrightarrow CO_3^{-2} + H^+ \quad \text{and} \quad HPO_4^{-2} \longrightarrow PO_4^{-3} + H^+ \text{-------(6.2)}$$

Chemical analysis and change in pH during experiment of the SBF solutions after immersion period of 1 day to 21 days are shown in Fig.6.5. It is observed that silica, PO_4^{-3}, and Ca^{+2} leach out from the surface. After one week of immersion, the concentrations of Si, Ca and P have increased slightly when compared to prepared SBF concentration. Amount of Si leaching out from the surface increasing slowly, as the Fe_2O_3 concentration increased from 2.5 to 10 wt.% and cobalt Oxide from 1.0 to 2.5 wt.%. The pH of the SBF solutions was also monitored during the experiment. The pH was found to increase from 7.4 to 9.92. This change in basicity is sharp from 7.4 to 9.84 (up to 1 week) and then gradual from 9.8 to 9.92 for (1–3) weeks.
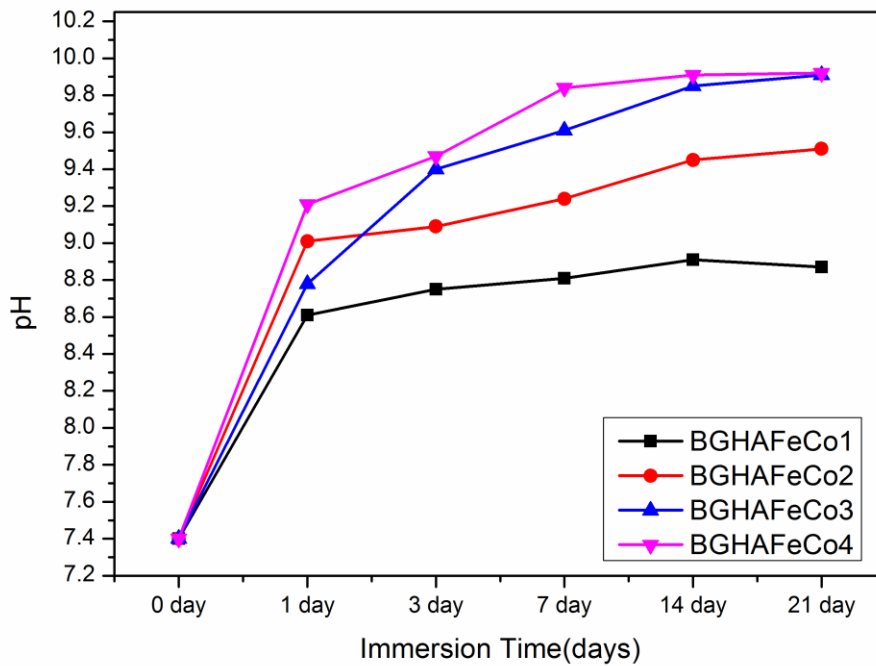
Figure 6.5 pH behaviour of the SBF after immersion of the biocomposite samples.

6.3.6 SEM and EDX analysis of biocomposites

The composite bone was subjected to the in vitro bioactivity test (soaking in SBF) to evaluate their ability to induce the precipitation of HA on their surface. SBF is an inorganic solution (which does not contain proteins, cells, and other organic moieties) having the same ionic composition of human blood plasma. This test is commonly used to assess the bioactivity of biocomposite, and it has been reported that the in vitro hydroxyapatite formation after immersion in SBF can be related to the in vivo bioactivity of biomaterials [T. Kokubo et al. 2006; C. Wu et al. 2009].
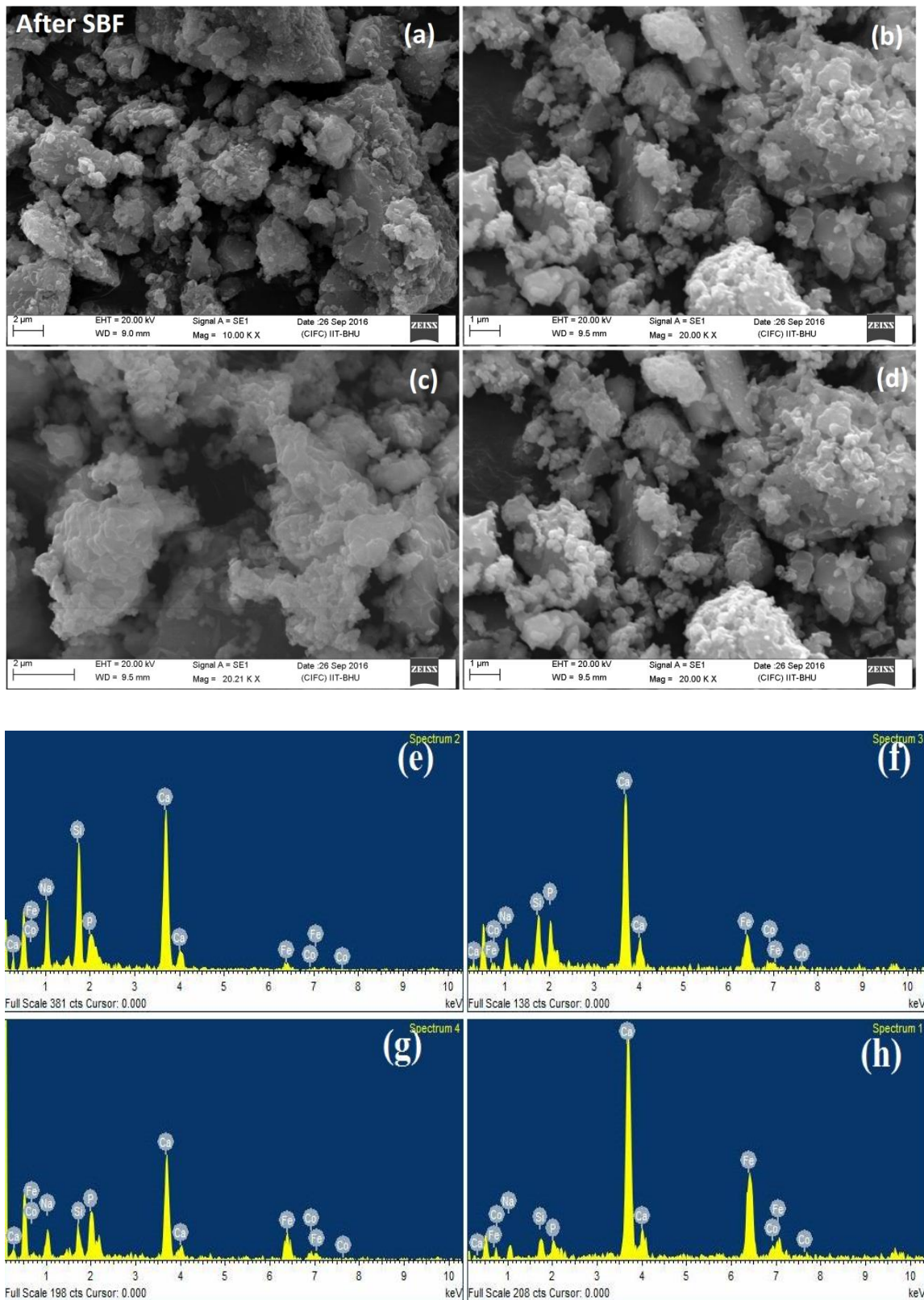
Figure 6.6 SEM of biocomposite samples after SBF (a-d) and EDX of biocomposite samples (e-h).

Fig. 6.6(a-d) shows the SEM micrographs of the biocomposite sample BGHAFeCo(1-4) after immersion in SBF for 21 days respectively. The micrographs have been obtained under 1000 magnification. The micrographs provide visual evidence of the formation of a surface layer on the biocomposite, which can now be presumed to be an appetite layer. After 21 days of immersion, the whole surface of the specimen is covered with spherical Ca-P particulate apatite layer. Energy dispersive spectrometer (EDS) composition analysis reveals the gradual development of hydroxyl carbonate apatite on the surface of biocomposite samples after immersion for various time periods in SBF. The spherical particles observed in the sample treated in SBF for 21 days are made up of calcium and phosphorus with the Ca/P molar ratio (calculated from EDS analysis) of 1.68, which is close to the value in HA. Microanalysis of the precipitates reveals the presence of small quantities of Na and Cl as shown in the EDS spectra in Fig. 6.6(e-h). This finding is an agreement with reports which claim that the growth of HA in SBF is accompanied by the incorporation of sodium, magnesium and chloride ions as well [S.V. Dorozhkin et al. 2003].

6.3.7 Mechanical Properties of biocomposites

The results of the compressive strength and density for BGHAFeCo1, BGHAFeCo2, BGHAFeCo3 and BGHAFeCo4 samples are shown in Fig.6.7. The increasing percentage tendency of the Fe_2O_3, CoO results demonstrate an increasing compressive strength (60, 72, 91, 109 MPa) and density (2.67, 2.75, 2.78, 2.87 g/cm^3) respectively. It is attributed to the reason that lighter element silicon has been replaced by heavier element iron and cobalt substitution of Fe_2O_3 (2.5 to 10 wt%) and cobalt Oxide (1 to 2.5wt%) in the bioactive glass samples. Azevedo et al. had also performed the 29Si MAS-NMR to determine the role of Co^{+2} ion in the silicate glass structure and reported

the dual role of Co^{+2} as an intermediate and modifier in their biocomposite samples depending upon its concentration like Mg^{+2}, Ca^{+2} and Zn^{+2} ions. This study is used for nonload bearing implant inside the human body [M.M. Azevedo et al. 2010].
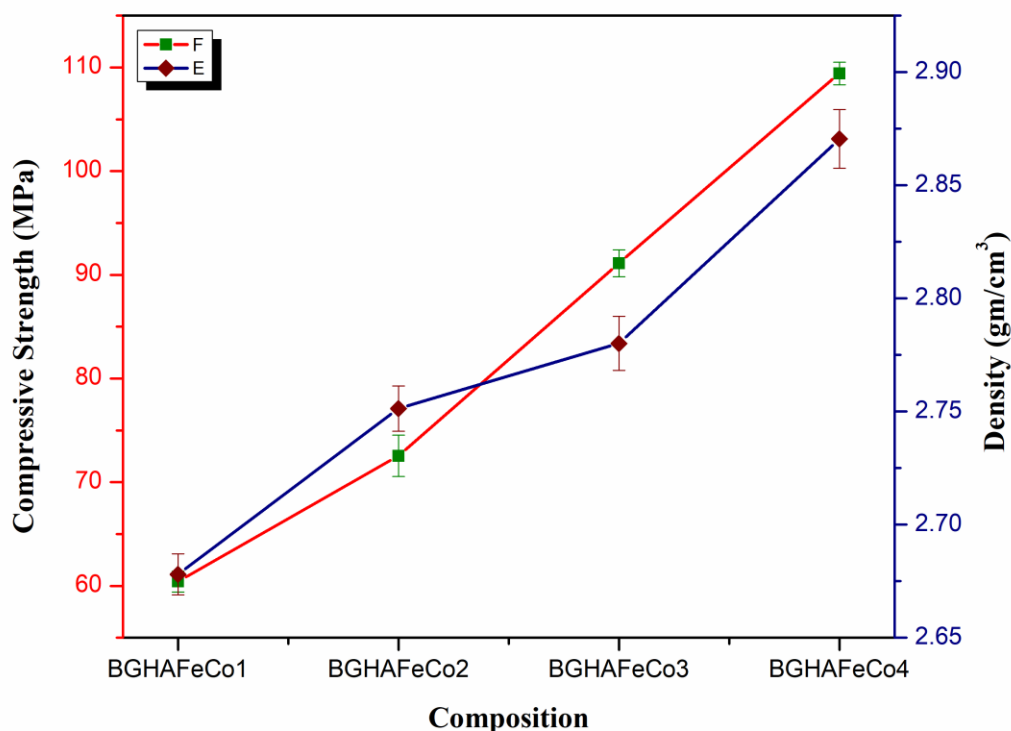

Figure 6.7 Compressive strength and density of biocomposites (BGHAFeCo1, BGHAFeCo2, BGHAFeCo3 and BGHAFeCo4) sample.

6.4 Conclusion

A magnetic bioactive glass-based biocomposite was synthesized by mixing Fe_2O_3 and CoO to bioglass 45S5-HA. Under a magnetic field up to saturation, the saturation magnetization, coercive force and magnetic hysteresis loss of the samples varied at 0.018 emu/g, 191.775 Oe, and 4.012 k erg/g, respectively. The mixing of Fe_2O_3 and CoO to bioglass 45S5 biocomposite was likely to decrease bioactivity. FTIR transmission spectra, pH behavior, XRD and SEM images indicated the formation of

hydroxyl carbonate apatite layer on the surface of the Fe_2O_3 and cobalt oxide containing bioactive composites after immersion in simulated body fluid. Beginning time of the HCA layer formation on 45S5 was 3–7 days while 14 days are required for an apatite layer to be formed on the surface of the BGHAFeCo biocomposite. The result shows that the most favorable sample for hyperthermia treatment of bone cancer are BGHAFeCo4 with magnetic hysteresis loss (4.012 k erg/g) and the onset time of the hydroxyapatite layer formation are 14 days. The density and compressive strength of biocomposites were enhanced with an increase in Fe_2O_3 and CoO content in the base bioactive glass powder.

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