CHAPTER-5

Destructive & non-destructive properties of cobalt oxide substituted 1393 bioactive glass

5.1 Introduction

Bioactive glasses (BGs) such as "45S5 Bioactive glass" ($45SiO_2$-24.5CaO-$24.5Na_2O$-6 P_2O_5) wt% [L. L Hench et al. 1991] and "1393 Bioactive glass" ($54.68SiO_2$-$5.98Na_2O$- $7.74K_2O$-7.74MgO-22.11CaO-$1.79P_2O_5$) mol% [M. N Rahaman. D. E Day, B. S Bal, Q. Fu, S. B Jung, L. F. Bonewald, and A. P Tomsia, 2011], compositions have been broadly used for bone tissue engineering applications. Apart from the aforementioned silicate glasses, borate and borosilicate glasses have now days found varying usage in biomedical and technological applications [Y. Yun, P. Bray, 1978, and T. Kokubo, H. Takadama, 2006]. On being exposed to biological conditions, chemical degradation occurs in the BG, leading to the formation of hydroxyapatite (HA) layer, which facilitates bonding between bones and tissues [W. Cao and L.L. Hench, 1996]. BG's possess superior biocompatibility but owing to their weak mechanical strength and stability, avenues for their use as porous scaffolds were limited; hence researchers' devised new strategies of ion doping for enhancing their biological performance. Among the prior mentioned BG's borate-based glasses have a better controllable rate of degradation to form HA than silicate glasses [H. Fu, Q. Fu, N. Zhou,W. Huang, M.N. Rahaman, D.Wang, and X. Liu 2009 and T. Furukawa and W.B. White, 1997] which makes them promising materials for future. Recent advances in the field of research in silicate-based BG have to lead to newer compositions of BG by the incorporation of ions like cobalt, silver, zinc, magnesium, copper, strontium, fluoride, boron, etc. in the silicate network [A.R. Boccaccini et al. 2014, M. Bellantone et al. 2002, S. Haimi, et al 2009, M. Diba et.al 2012, H.Wang et al 2016, J. Lao et al. 2009, E. Gentleman, et al, 2013 and X. Liu, et al. 2009]. These ions can be slowly released in the human body in a controlled manner on scaffold degradation. These new glasses are highly beneficial as they help in the process of osteogenesis and angiogenesis and act as

an anti-bacterial agent in chemical applications of engineered bone constructs [H. Pan et al. 2010, D. Clupper et al. 2001, C.Wu et al. 2011, H. Wang et al. 2014, W. Hui et al. 2014]. They are of importance as they can be used for the limited delivery of certain ions which control particular cell functions [A. Hoppe, N. S. Geuldal, and A. R. Boccaccini 2011], carrier of homeostatic agents [T. A. Ostomel, Q. Shi, C. K. Tsung, H. Liang, and G. D. Stucky, 2006] and therapeutic drugs [J. Hum, and A. R. Boccaccini 2012], hyperthermia treatment of cancer [O. Bretcanu, S. Spriano, C. B. Vitale, and E. Verne 2006] and also for soft tissue applications like nerve generation [X. F. Zhang, H. O'Shea, S. Kehoe, and D. Boyd,2011]. 1393 bioactive glasses tolerate strong bonding to hard and soft tissue and have been shown to endorse osteogenesis via the activation of several relevant genes [L. L Hench et al. 2009]. Cobalt ions are considered important in bone physiology [R. Buttyan et al. 2003, S. Patntirapong et al. 2009, K. Peters et al. 2002] as they induce hypoxia conditions and become stable hypoxia-inducible factor [G. Chachami et al.,2004, K. Peters et al. 2005] which in turn, activates several pro-regenerative processes in the human body [G.L. Semenza, 2007]. HIF-1 activation leads to angiogenesis, stem cell differentiation, and fracture repair [M.M. Azevedo et al. 2010, P.J. Emans et al. 2007]. Cobalt releasing bioactive glasses is thus called hypoxia-mimicking [M.M. Azevedo et al. 2010] and has potential benefits in vivo. A higher concentration of Co can prove toxic [C. Fleury, A. Petit, F. Mwale, J. Antoniou, D.J. Zukor, M. Tabrizian, and O.L. Huk, 2006 M. S. Gaafar, S. Y. Marzouk, H. A. Zayed, L. I. Soliman, and A. H. Serag El-Deen 2013] and hence, it needs to be released in a controlled manner in the physiological environment. In the present work, however, the mechanical properties of Co substituted 1393 bioactive glass have been studied to assess its suitability for various biological applications.

5.2 Material and methods

5.2.1 Preparation of bioactive glasses

Four batches having the concentration of raw materials were taken to prepare the glass samples. Normal melting and annealing techniques were used to prepare bioactive-glasses. For SiO_2, quartz with fine-grained was taken. Soda and lime were introduced in the form of their respective anhydrous carbonates (Na_2CO_3 and $CaCO_3$). $P_2O_5$was added in the form of ammonium dihydrogen orthophosphate ($NH_4H_2PO_4$). MgO and K_2O were also incorporated in the form of their respective carbonates ($MgCO_3$ and K_2CO_3). Cobalt Oxide was available as CoO. The wt % of the different bioactive glass samples composition is given in **Table 5.1**. These raw materials were mixed thoroughly for 30 minutes in an already cleaned mortar and pestle. Different batches were kept in alumina crucibles and placed in an electric furnace to melt at a temperature of 1400±5°C for 3 hours. This steady-state was maintained for another hour after which the homogenized melts were poured onto a steel mold and transferred to a regulated electric furnace already at a temperature of 470^{o}C. The process of annealing was carried out for an hour after which the furnace was allowed to cool down to room temperature at a controlled rate of 100°C/h. Glasses are annealed to remove any internal stresses which developed during the formation process to prevent them from cracking on the application of mechanical or thermal shocks.

Table 5.1- Composition of base and doped bioactive glasses (wt %)

	SiO_2	**Na_2O**	**Cao**	**P_2O_5**	**CoO**	**K_2O**	**MgO**
1393	53.00	6.00	20.00	4.00	0.00	12.00	5.00
Co-1	52.50	6.00	20.00	4.00	0.50	12.00	5.00
Co-2	52.00	6.00	20.00	4.00	1.00	12.00	5.00
Co-3	51.50	6.00	20.00	4.00	1.50	12.00	5.00
Co-4	51.00	6.00	20.00	4.00	2.00	12.00	5.00

5.2.2 X-ray diffraction measurements

The bioactive glass samples were ground to powder form having a grain size of 75μm for X-ray diffraction analysis. XRD is done in order to distinguish and identify the crystalline phases present in the glass, which itself is amorphous. A RIGAKU-Miniflex II diffractometer adopted Cu-Kα radiation (λ=1.5405 Å) having a tube voltage of 40kV and current of 35mA was used in the 2Θ range between 20^o and 80^o. The step size was set to 0.02^o, and the measuring speed was 1^o per minute. JCPDS data cards were used as a reference for identifying the peaks in the graph.

5.2.3 Structural analysis using FTIR spectrometry

FTIR spectrometry is carried out in order to determine the functional groups present in the bioactive glasses. For this, the powdered glass samples were mixed with KBr in the ratio of 1:100 respectively and were put inside an evocable die to form homogenous discs by applying a load of 10MPa. As KBr is prone to moisture attack, the discs were immediately subjected to an IR spectrometer (VARIAN Scimitar 1000, USA) for measuring the absorption spectra. The analysis was done at room temperature within the wave number range of 4000-400 cm^{-1}.

5.2.4 Density measurement

The densities of all samples were measured at room temperature using a digital balance (Satorius, Model- BP221S, USA) which has an accuracy of ±0.0001 gm. Water was used as an immersion fluid. Density was determined by Archimedes principle using the following formula.

$$\text{Density} = [W_a / (W_a - W_w)] \times 0.988 \text{ gm/cc}$$

Where, 'W'_a is the weight in air, 'W_w' is the weight in water and density of water is 0.988 gm/cc.

5.2.5 Measurement of mechanical properties

Flexural strength

The bioactive glass samples were cast in cubical shape and were ground and polished to get the desired size of 1cm x 1cm x 1cm. These samples were subjected to three point bending test at room temperature using an Instron Universal Testing Machine (AGS 10kND, SHIMADZU) whose cross- head speed was 0.5 mm/min bearing a full scale load of 2500 kg. The calculation of flexural strength was calculated using the formula.

$$F = (3P_f L) / (2bh^2)$$

Where 'P_f' is the load and 'L', 'b', 'h' are the length, breadth and height of the glass samples respectively.

Compressive Strength

For measuring compressive strength of the bioactive glass samples the Instron Universal Testing Machine was used having a cross-speed of 0.05 cm/min and full scale load of 2500 kg. The samples were cut into the desired size of 2cm x 2cm x 1cm according to ASTM standard D3171 and the test was conducted at room temperature

Hardness-The hardness of specimens having size of 1cm x 1cm x 1cm according were mesured to ASTM standard C730-98 using the Hardness Testing Machine. Indentations were made on the samples with loads lying within the range of 30mN - 2000mN which were applied at a velocity of 0.1 cm/sec. Formula for calculation of micro hardness (GPa) is given as belong.

$$H = 1.854 \times (P/d^2)$$

Where 'P' is the load and'd' is the diagonal of the indentation.

Elastic properties

The 1393 and Cobalt doped glass samples were cut and polished into cubic pieces and placed inside the instrument Olympus (M-45, USA) for measuring the ultrasonic wave velocities. Sonfech shear gel and Couplant glycerin were used for measuring the shear and longitudinal wave velocities, respectively. Various formulae involving these velocities and density values were used for the calculation of Poisson's Ratio, Young's, Shear and Bulk Modulus of elasticity.

5.3 Results and discussion

5.3.1 X-ray analysis of bioactive glasses

The XRD patterns for an un-doped and Co-doped 1393 bioactive glass samples are shown in Figure. 5.1. The results obtained are in good agreement with the fact that glass is an amorphous nature, and hence the Figure is devoid of peaks which indicates the absence of any crystalline phase. The hump for 2Ɵ values between 25^{o} and 35^{o} intensifies with the addition of cobalt oxide and is the only change visible in the different graphs. It also shows that cobalt oxide is totally dissolved in the glass matrix.

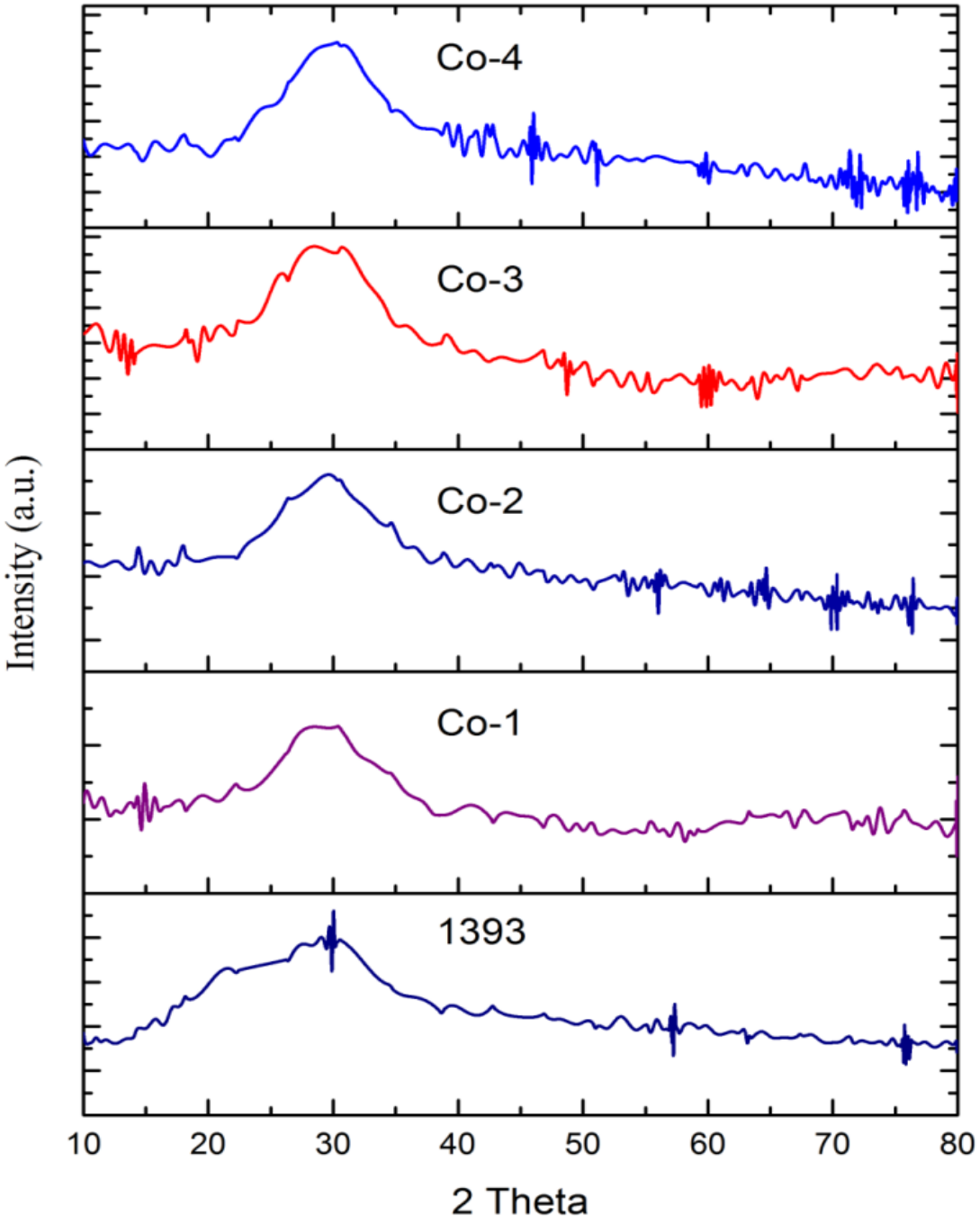

Figure 5.1- XRD of base & substituted bioactive glasses

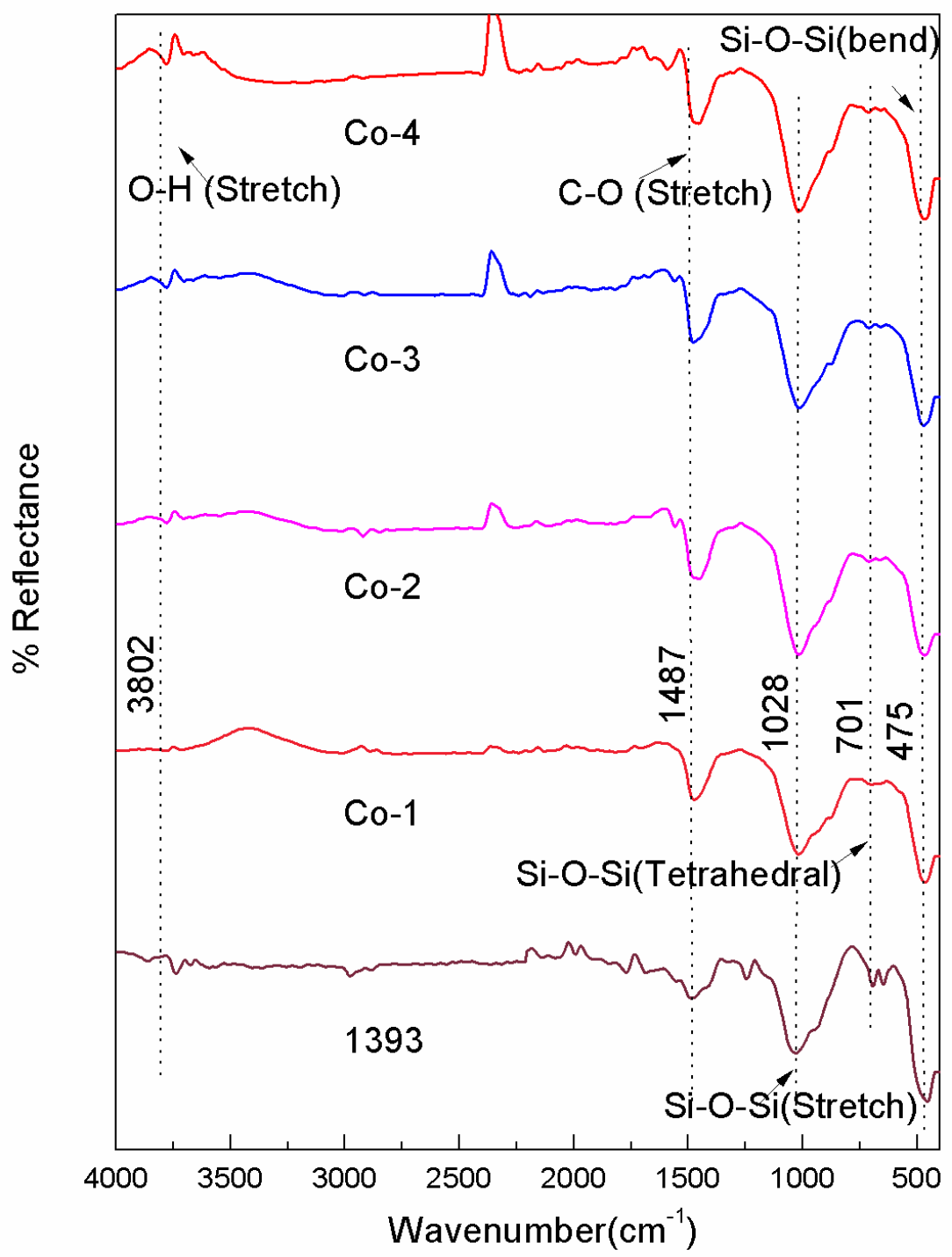

Figure 5.2- FTIR of base & substituted bioactive glasses

5.3.2 FTIR spectra of bioactive glasses

Figure 5.2 shows the FTIR Reflection spectra of base 1393 bioactive glass and Co substituted 1393 bioactive glass. The presence of the silicate network is confirmed

from the obtained spectra bands due to SiO_2 being the major constituent. Peaks can be seen at 475, 701, 1028, 1487, and 3802 cm^{-1}. The peak at 475 cm^{-1} can be connected with Si-O-Si symmetric bending mode, and the one at 701 cm-1 corresponds to an asymmetric stretch of non-bridging oxygen atoms. The main band at 1028 cm^{-1} can be attributed to Si-O-Si stretching mode. The small band at 1487 cm^{-1} can be assigned to the C-O vibration mode. Finally, the small and broadband at 3802 cm^{-1} can be associated with the hydroxyl group (-OH), which indicates the presence of adsorbed water molecules. There is no noticeable change in the IR spectra bands in the cobalt oxide substituted 1393 bioactive glass.

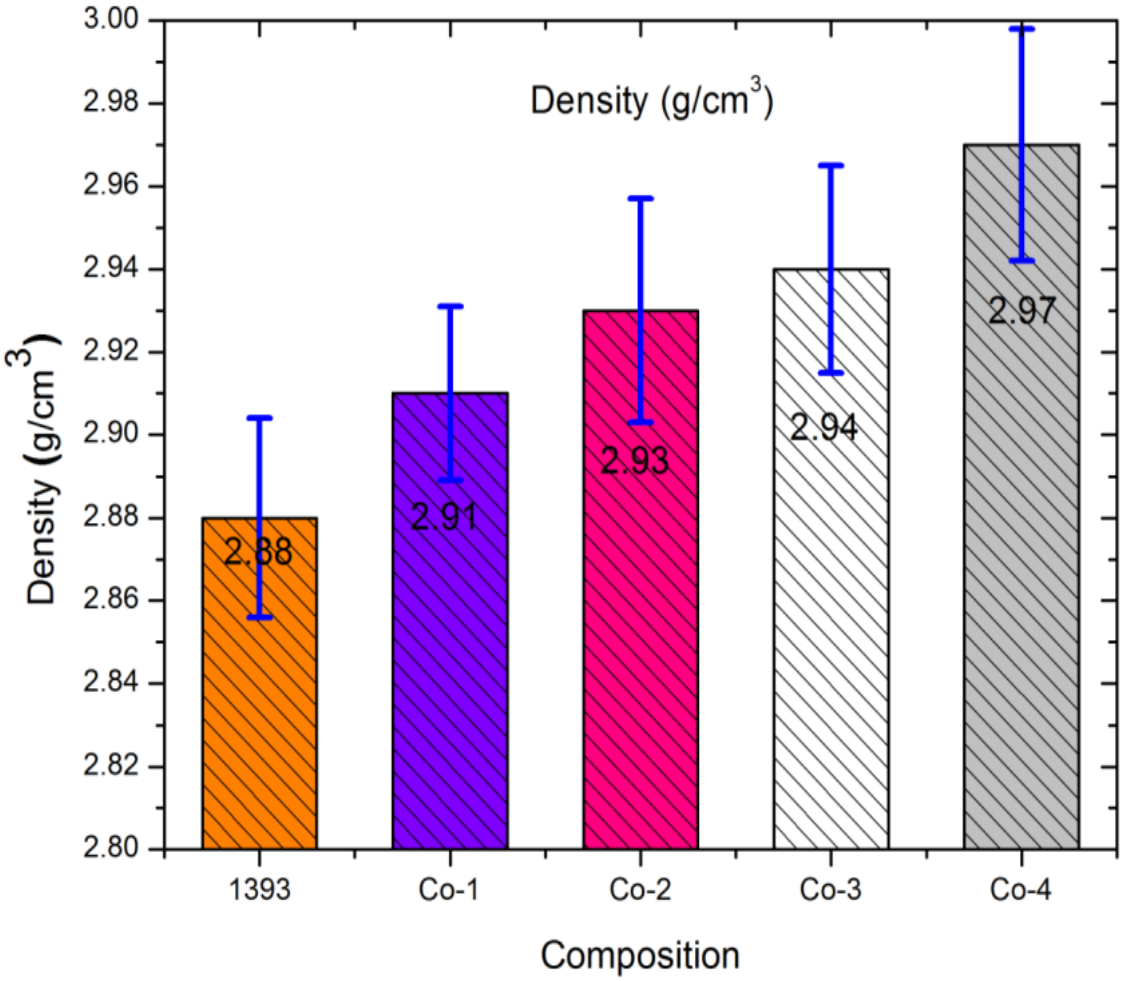

Figure 5.3- Density of base & substituted bioactive glasses

5.3.3 Density measurements and mechanical properties of doped and undoped 1393 bioactive glasses

The variation in density measurements calculated using Archimedes' principle is depicted in **Figure 5.3** which clearly indicated that the addition of cobalt oxide makes the glass denser and therefore the density gradually increases from 2.88 to 2.97 g/cm^3. This increase can be ascribed due to partial replacement of lighter element SiO_2 having density 2.65 g.cm^{-3} with heavier cobalt oxide having density 6.11 g.cm^{-3}. This is due to reduction in average interatomic Spacing in the bioactive glasses, which coordinated the NBOs and formed the interlink between the atoms, resulting in an increase in connectivity of network dimensionality. Thus it leads to efficient compactness packing in the structure.

The flexural strength (**Figure.5.4**) of 1393, Co-1, Co-2, Co-3, Co-4 bioactive glass was measured as 44.46, 57.24, 58.41, 62.49, 66.55 MPa and compressive strength (Figure.5) as 69.82, 78.63, 81.35, 79.15, 84.13 MPa respectively. Micro Hardness (Figure.6) values on calculation came out to be 5.45, 5.58, 5.61, 5.87, 6.09 GPa for the aforementioned glasses respectively. All of them show an increasing trend on subsequent addition of cobalt oxide. Cobalt acts as a network intermediate when added in quantities less than 2 wt% and as a modifier when its concentration lies between 2 and 4 wt% [M.M. Azevedo, G. Jell, M.D. O'Donnell, R.V. Law, R.G. Hill and M.M. Stevens 2010]. As in the present study there has been a gradual addition of 0-2.00 wt% of Co^{2+} ions it acted as an intermediate and made the glass structure more compact. This justifies the changing trend in the mechanical properties.

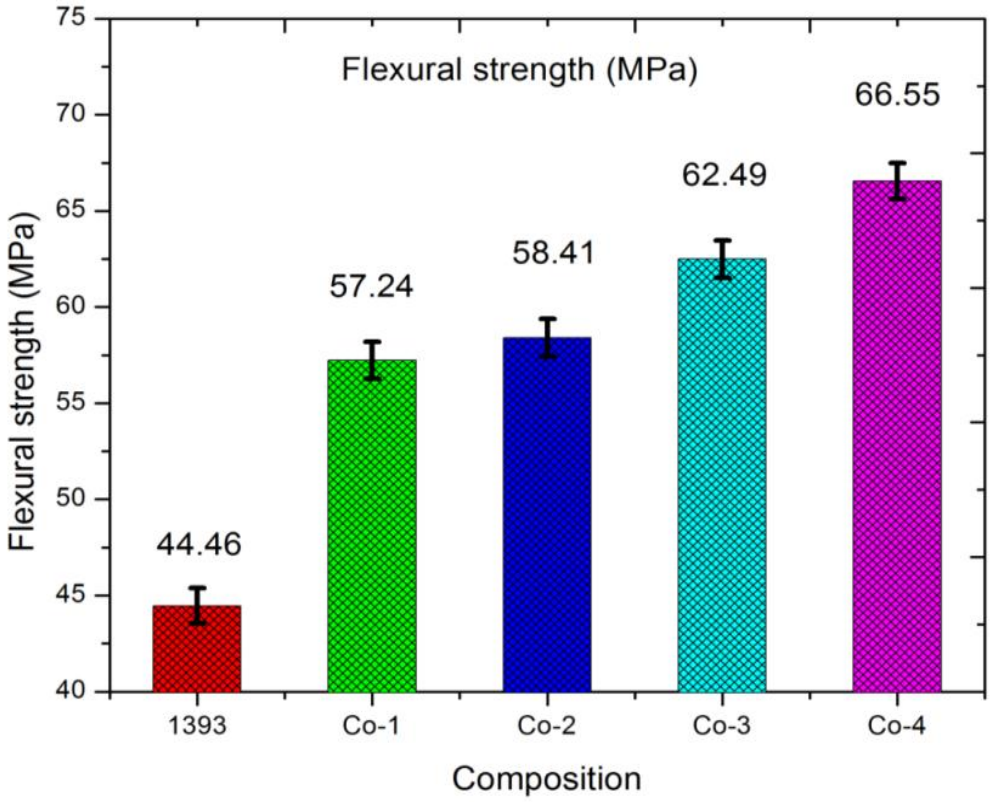

Figure 5.4- Flexural strength of base & substituted bioactive glasses

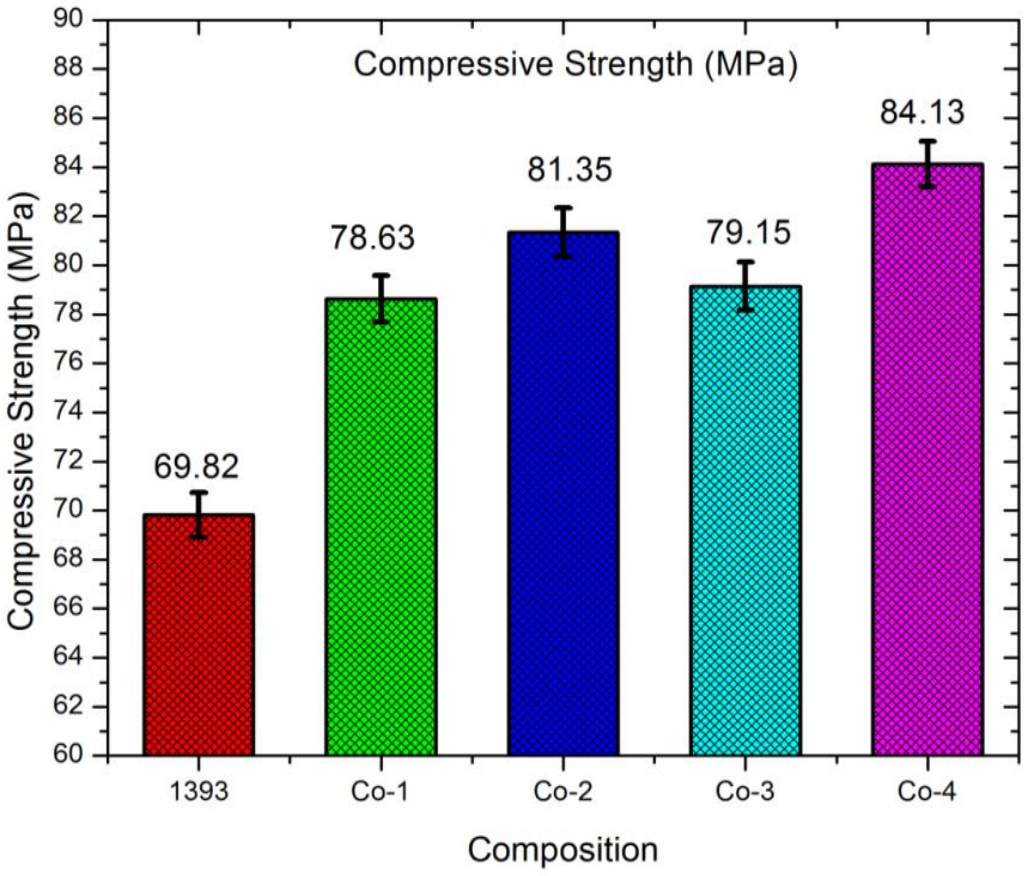

Figure 5.5- Compressive strength of base & substituted bioactive glasses

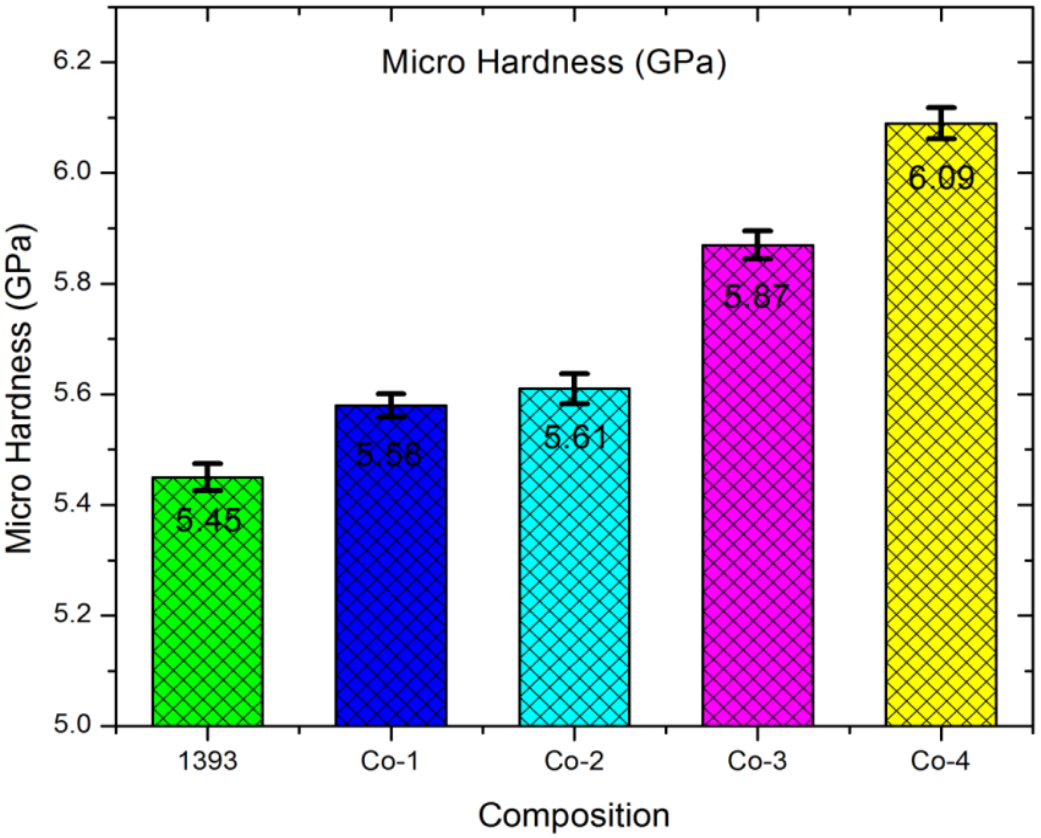

Figure 5.6- Micro hardness of base & substituted bioactive glasses

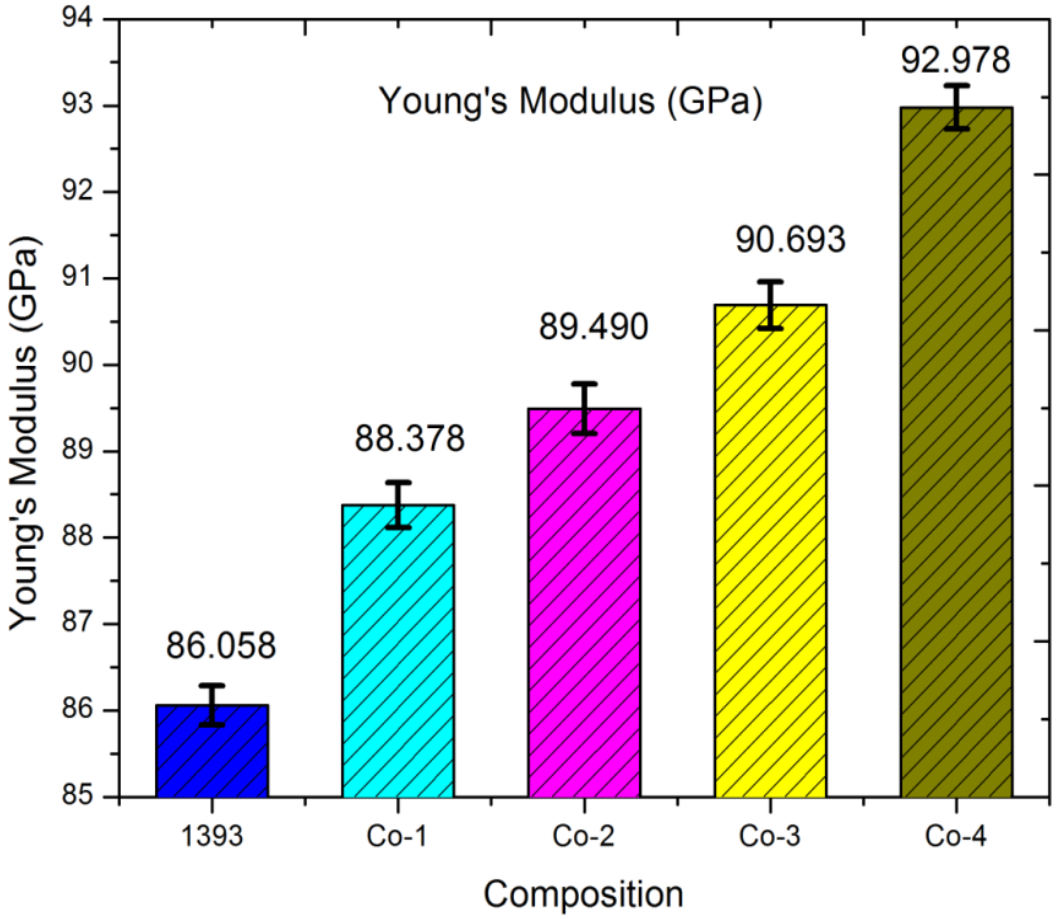

Figure 5.7- Young's modulus of base & substituted bioactive glasses

5.3.4 Elastic properties

The elastic properties, namely Poisson's Ratio, Young's, shear, and Bulk modulus of glasses, were calculated using the longitudinal and shear ultrasonic wave velocities. These values were graphically represented in **Figure. 5.7 to 5.10**

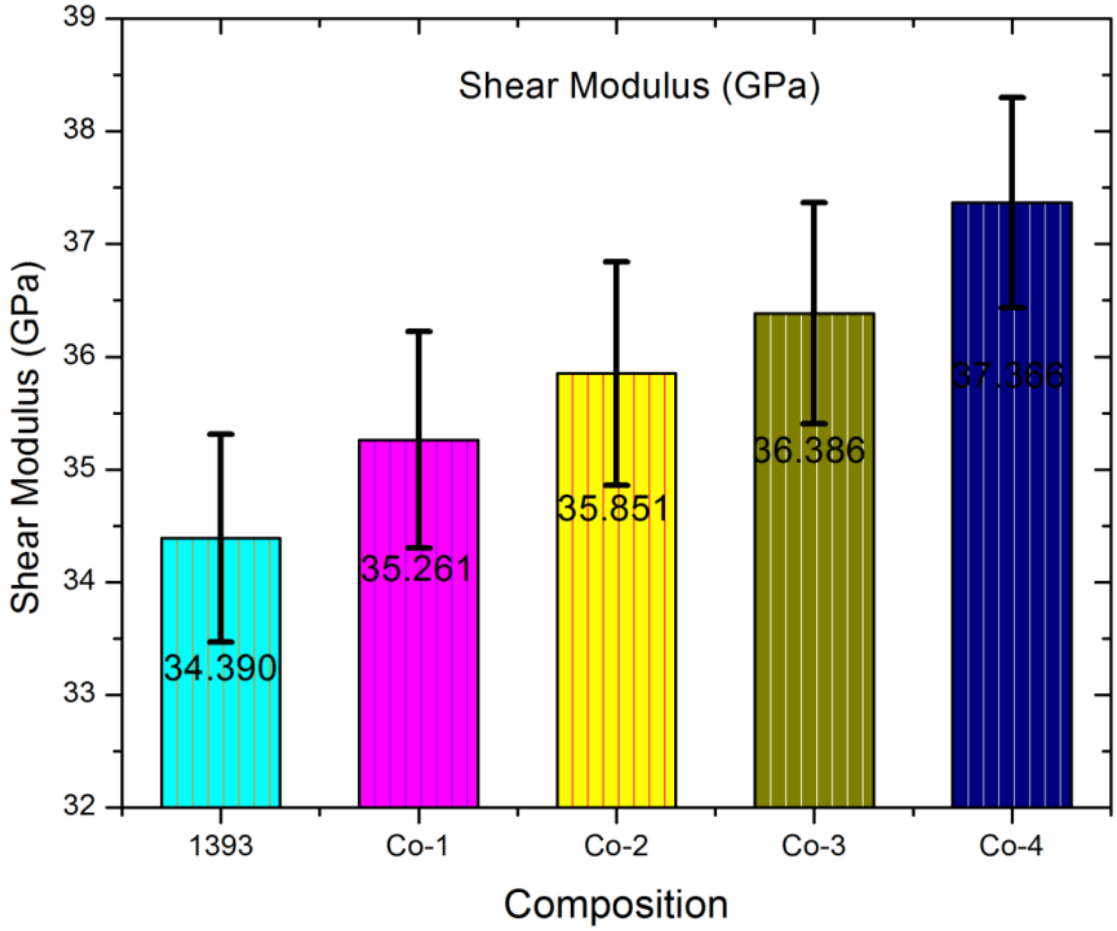

Figure 5.8- Shear modulus of base & substituted bioactive glasses

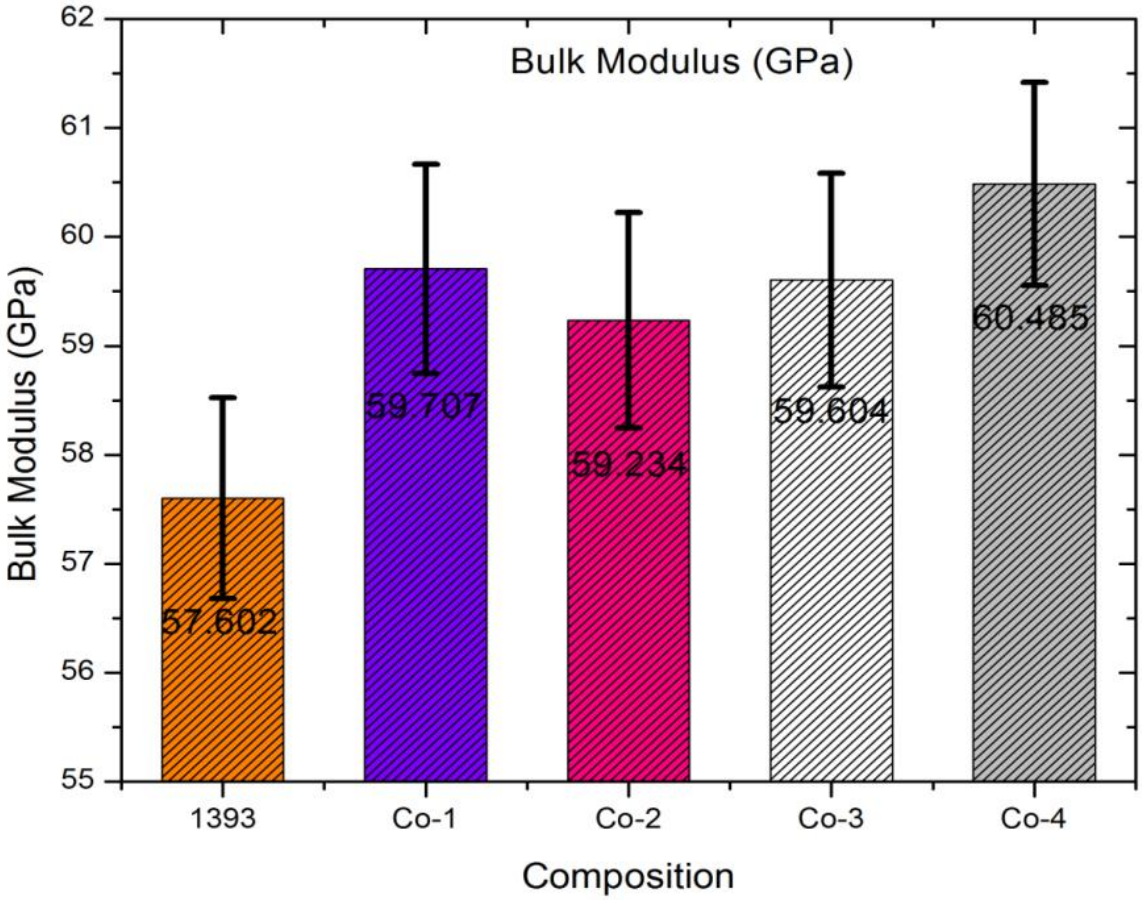

Figure 5.9- Bulk modulus of base & substituted bioactive glasses

There is an increase in values of Young's, shear, and bulk modulus with an increase in Co^{2+} concentration whereas a slightly decreasing trend in values is observed for Poisson's ratio. Increase in elastic modulus values has been earlier reported by [Gaafar and Kannapan 2013] which was ascribed to the increase in connectivity in the glass network. However, the slight reduction in Poisson's ratio could have been due to change in the type of bonding in the glass structure on the addition of cobalt oxide [Gaafar et al. 2013].

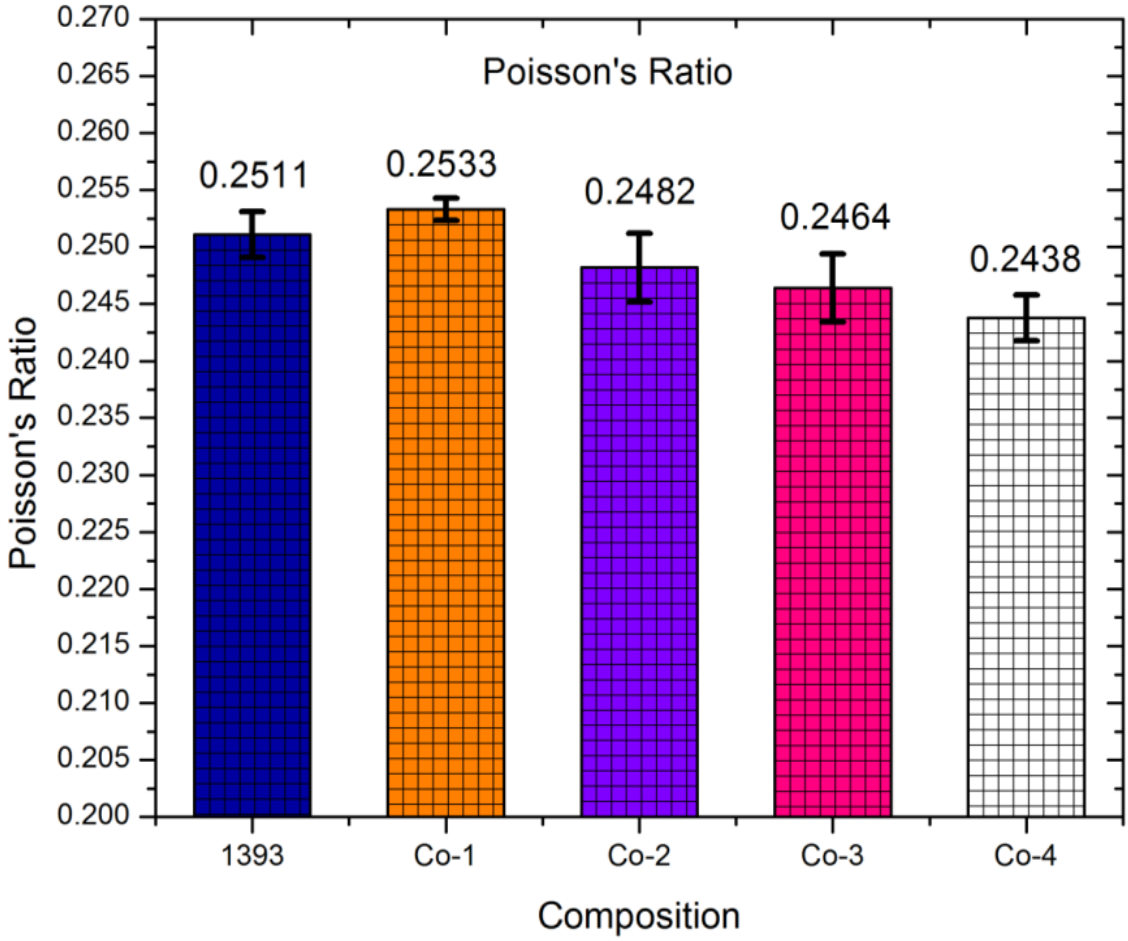

Figure 5.10- Poisson's ratio of base & substituted bioactive glasses

5.4 CONCLUSIONS

In the present investigation, various types of properties were carried out in 1393, Co-1, Co-2, Co-3, Co-4 substituted bioactive glass samples. Firstly, the XRD analysis confirmed the amorphous nature of glass, and FTIR result showed the presence of the silicate network in the glass structure. The study of mechanical properties, namely bending and compressive strength and hardness and elastic properties namely Young's, bulk and shear moduli show an upward trend on increasing the concentration of CoO in the bioactive glass. However, the values for Poisson's ratio reduce slightly on Co^{2+} addition. On the one hand, as the doped bioactive glass emerges to be mechanically stronger than the base glass but on the other hand, it is structurally weaker in due to the

replacement of stronger Si-O-Si bonds with Co-O-Si bonds. Therefore on summarizing the results obtained from investigation, it can be concluded that cobalt oxide substituted 1393 bioactive glass can be used in biomedical applications as a potential biomaterial.