CHAPTER-2

Material and methods

2.1 Selection of composition

The 1393 bioactive glass composition was formulated from $53SiO_2$-$6Na_2O$-$12K_2O$-$5MgO$-$20CaO$-$4P_2O_5$ glass system. First, the 1393 bioactive glass, having wt% composition [$53SiO_2$-$6Na_2O$-$12K_2O$-$5MgO$-$20CaO$-$4P_2O_5$] was prepared. Then the proposed bioactive glass containing chemical composition (having wt% composition)

(53-X)SiO_2-XTiO_2-$6Na_2O$-$12K_2O$-$5MgO$-$20CaO$-$4P_2O_5$ (where X= 0-2 wt%)

(53-X)SiO_2-XCoO-$6Na_2O$-$12K_2O$-$5MgO$-$20CaO$-$4P_2O_5$ (where X= 0-2 wt%)

(53-X)SiO_2-XZrO_2-$6Na_2O$-$12K_2O$-$5MgO$-$20CaO$-$4P_2O_5$ (where X= 0-2 wt%)

$53SiO_2$-XZrO_2-$6Na_2O$-$12K_2O$-$5MgO$-(20-X)CaO-$4P_2O_5$ (where X= 0-2 wt%)

In the bioglass composition, the role of SiO_2 is as a glass former while CaO, Na_2O, K_2O, MgO treated as network modifier and P_2O_5 is network former. In this study the weight percent of Na_2O (Merck, India), K_2CO_3 (Merck, India), $MgCO_3$ (Merck, India) and P_2O_5 (Merck, India), was kept constant and SiO_2 was partially replaced with TiO_2, (Merck, India) CoO (Merck, India) and ZrO_2 (Merck, India) and CaO (Merck, India) was partially replaced with ZrO_2 (Merck, India).

2.2 Bioactive glass preparation

The compositions of bioactive glass as given in Table-2.1 were prepared by substitution of TiO_2, CoO and ZrO_2 in place of SiO_2 and CaO using the normal melting and annealing technique. Materials used include fine-grained quartz (Merck, India) for silica. Lime and soda were introduced in the form of their respective anhydrous carbonates i.e. K_2CO_3 (Merck, India) and $MgCO_3$ (Merck, India). P_2O_5 was introduced in the form of ammonium dihydrogen orthophosphate [$NH_4H_2PO_4$] (Merck, India). All the materials were of analytical grade chemicals and used without further purification. The weighed batches were mixed using agate mortar and pestle thoroughly for 40 minutes and melted in alumina crucibles of 100 ml capacity. The melting was carried

out in an electric furnace at 1400±5°C for 3 hours in the air as furnace atmosphere and homogenized melts were poured on preheated aluminum sheet. The prepared bioactive glass samples were directly transferred to a regulated muffle furnace at 470 °C for annealing. After 2 h, the annealing furnace was cooled to room room temperature at the rate of 100^0C/hr.

Table 2.1- Composition of TiO_2, CoO and ZrO_2 bioactive glasses (wt %)

	SiO_2	**Na_2O**	**CaO**	**P_2O_5**	**TiO_2**	**K_2O**	**MgO**
1393	53.00	6.00	20.00	4.00	0.00	12.00	5.00
TiO_2-1	52.50	6.00	20.00	4.00	0.50	12.00	5.00
TiO_2-2	52.00	6.00	20.00	4.00	1.00	12.00	5.00
TiO_2-3	51.50	6.00	20.00	4.00	1.50	12.00	5.00
TiO_2-4	51.00	6.00	20.00	4.00	2.00	12.00	5.00

	SiO_2	**Na_2O**	**CaO**	**P_2O_5**	**ZrO_2**	**K_2O**	**MgO**
Zr-0	53.00	6.00	20.00	4.00	0.00	12.00	5.00
Zr-1	52.50	6.00	20.00	4.00	0.50	12.00	5.00
Zr-2	52.00	6.00	20.00	4.00	1.00	12.00	5.00
Zr-3	51.50	6.00	20.00	4.00	1.50	12.00	5.00
Zr-4	51.00	6.00	20.00	4.00	2.00	12.00	5.00

	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

	SiO_2	**Na_2O**	**K_2O**	**P_2O_5**	**MgO**	**CaO**	**ZrO_2**
1393	53	6	12	**4**	**5**	20.0	0.0
G-1	53	6	12	4	5	19.5	0.5
G-2	53	6	12	4	5	19.0	1.0
G-3	53	6	12	4	5	18.5	1.5
G-4	53	6	12	4	5	18.0	2.0

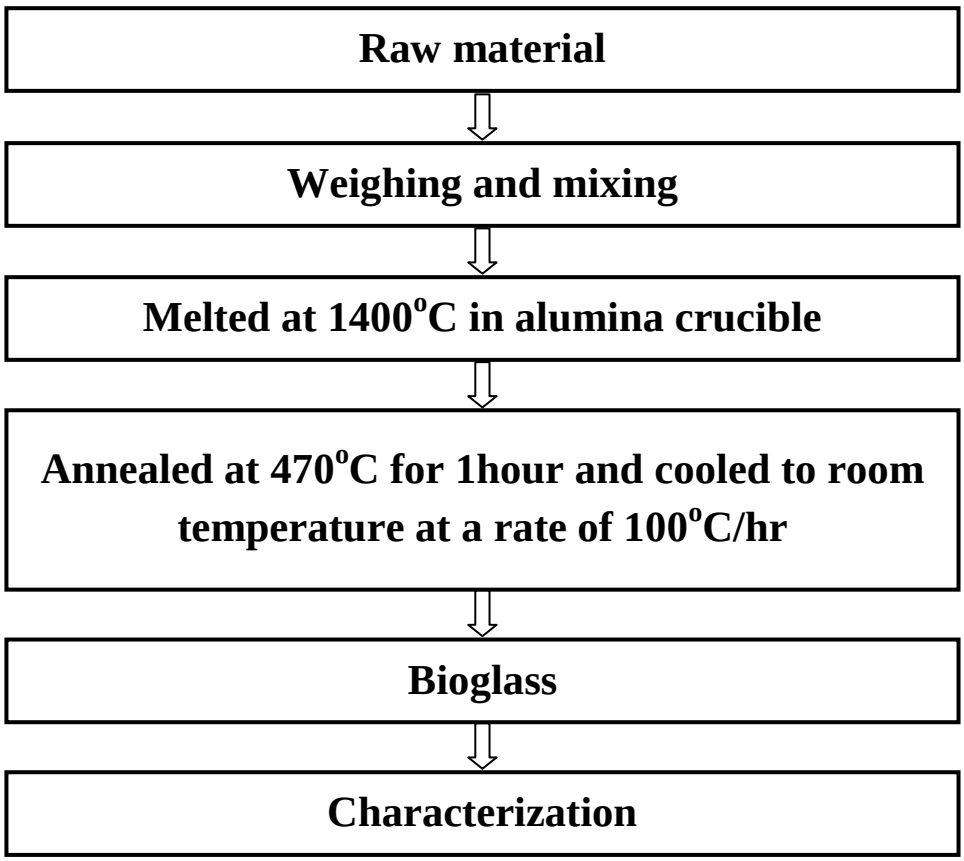

Figure 2.1- Flow chart - preparation of bioactive glass

Various reactions taking place during melting and annealing are:

$CaCO_3$ + heat ---> $CaO + CO_2\uparrow$ (910^oC at pressure 1 bar)

$MgCO_3$ + heat ---> $MgO + CO_2\uparrow$ (540^oC at pressure of 1 bar)

$SiO_2 + Na_2CO_3$ ----> $Na_2SiO_3 + CO_2$

$SiO_2 + K_2CO_3$ ----> $K_2SiO_3 + CO_2$

$SiO_2 + MgCO_3$ ----> $MgSiO_3 + CO_2$

$SiO_2 + CaCO_3$ ----> $CaSiO_3 + CO_2$

$2(NH_4)_2H_2PO_4$ ----> $P_2O_5 + 4NH_3 + 3H_2O$

2.3 Roles of SiO_2, CaO, Na_2O, MgO, K_2O and P_2O_5 in 1393 bioglass

SiO_2 :

Glass former provide viscosity to the glass and mechanical and thermal structural stability. A 40-60 wt% of SiO_2 is desirable for the glass to be

bioactive. The silica is gradually converted into HA depending upon the percentage of SiO_2 in bioglasses.

CaO :

Network modifier, Ca is a prerequisite element to form mineral composition of bone. Apart from network modification, it forms hydroxyapatite layer on the glass surface by coupling with phosphate ions in physiological fluid. As a network modifier, its incorporation increases the non bridging oxygen hence decreases the bond strength and thus lowers melting point. It incorporates the crystalline tendency in glasses. Selected for the high amount of CaO, this composition had also the advantage of being extremely easy to melt due to its proximity to the ternary eutectic.

Na_2O :

Network modifier, disrupt Si-O-Si tetrahedron and thus create non bonding oxygen. Increase in Na will cause decrease in mechanical strength and bioactivity but will increase crystalline properties in bio glasses.

MgO :

Provide mechanical strength, structural and thermal stability to the glasses, lessen biodegradation and retard bio mineralization. Magnesium is the fourth most abundant cation in the human body, being present in the natural enamel, dentin and bone. It plays an important role in bone metabolism. The addition of magnesium to the glasses in the SiO_2-CaO-Na_2O-P_2O_5 system influences the bioactivity.

K_2O :

Network modifier potassium provides strength and increases toughness of the glasses.

$\underline{P_2O_5}$:

Network former, phosphorus is elemental composition of bone minerals. High Ca/P ratio makes the glass surface very reactive to the physiological system.

2.4 Preparation of SBF

Kokubo and his colleagues developed simulated body fluid that has inorganic ion concentrations similar to those of human body fluid to reproduce in vitro formation of apatite on bioactive materials [Kokubo et al. 2006]. The SBF solution was prepared by dissolving reagent-grade NaCl, KCl, $NaHCO_3$, $MgCl_2.6H_2O$, $CaCl_2$, Na_2SO_4 and $KH_2PO_4.3H_2O$ into double distilled water and it was buffered at pH=7.4 with TRIS (tris hydroxymethyl aminomethane) and 1N HCl at 37°C as compared to human blood plasma (WBC). The ion concentrations of SBF in mM/liter of the solution are given in Table 2.2 [Kokubo et al. 2006].

Table 2.2- Ion concentration (mM/liter) of simulated body fluid and human blood plasma

Ion	Na^+	K^+	Mg^{2+}	Ca^{2+}	HCO_3^-	HPO_4^{2-}	SO_4^{2-}	Cl^-
Simulated body fluid	142.0	5.0	1.5	2.5	4.2	1.0	0.5	147.8
Human blood plasma	140.0	5.0	1.5	2.5	27.0	1.0	0.5	103.0

2.5 pH measurement

To measure the pH of bioactive glasses, 0.2 gram of bioactive glass powder was immersed in 20 ml of SBF solution at 37 ºC for a different time period, and the pH was measured using Universal Bio microprocessor pH meter. The instrument was calibrated each time with standard buffer solutions of pH 4.00, and 7.00 at room temperature and pH values have been measured for different time periods at a fixed time interval.

Figure 2.2- Universal bio microprocessor pH meter made by India (New Delhi)

2.6 X-ray diffraction analysis of powders

In order to identify the crystalline phase present in the heat-treated bioactive glasses the samples were ground to 75 microns and the fine powders were subjected to X-ray using RIGAKU-Miniflex II diffractometer adopted Cu-Kα radiation (λ = 1.5405A°) with a tube voltage of 40 kV and current of 35mA in a 2θ range between 20° and 80°. The step size and measuring speed was set to 0.02° and 1° per min, respectively was used in the present investigation. The JCPDS-International Centre for diffraction Data Cards were used as a reference.

Figure 2.3- X-ray diffractometer

2.7 In vitro test by FTIR reflectance spectrometry

The structure of bioactive glass was measured at the room temperature in the wave number range of 4000–400 cm^{-1} using a fourier transform infrared spectrometer, (Bruker Tensor 27 FTIR, Germany). The fine bioactive glass powder samples were mixed with KBr in the ratio of 1:100, and the mixtures were subjected to an evocable die at a load of 10 bar pressure to produce clear homogeneous discs.

Figure 2.4- FTIR reflectance spectrometer

The prepared discs were immediately subjected to FTIR spectrometer to measure the reflection spectra to avoid moisture attack. In order to investigate the formation of (calcium phosphate) apatite layer on the surface of the samples after immersion in SBF solution. 0.2 gram of the sample was immersed in 20 ml of SBF solution in a small plastic container at 37°C at the pH of 7.40 in an incubator at the static condition for periods 1, 3, 7, 15 and 30 days. After soaking, the samples were filtered, rinsed with double distilled water and dried in an air oven at 120 °C for 2 hours before FTIR spectrometric analysis.

2.8 Mechanical properties and density measurements

The melts were cast in a rectangular shaped mold, and the resultant glass samples were ground and polished for required dimension using grinding machine and then samples were subjected to three-point bending test. The test was performed at room temperature using Instron Universal Testing Machine (AGS 10kND, SHIMADZU) of the cross-head speed of 0.5 mm/min and a full-scale load of 2500 kg. Flexural strength was determined using the formula (1)[Chen, et al., 2006].

$$[\sigma_f = (3P_f L)/(2bh^2)] \text{ - - - - - (1)}$$

Where P_f is the load at which specimen being fractured, L is the length over which the load is applied, b is the width and h is height of sample.

Polished bioactive glass samples is prepared to measure the microhardness, using the hardness testing machine, the size of the sample was 10mm x 10mm x 10 mm according to ASTM Standard: C730-98. The indentations have been made for loads ranging between 30 mN and 2000 mN, applied at a velocity of 1 mm/s and allowed to equilibrate for 16 seconds before measurement. Microhardness, H (GPa) of 1 mm/s was calculated using the formula----- (2) [Michel, et al., 2004].

$$H = 1.854\ (P/d^2) \quad \text{- - - ----- (2)}$$

Where P (N) is the applied load on sample and d (m) is the diagonal of the impression

Compressive strength of the base glass, TiO_2, ZrO_2 and CoO doped bioactive glass (2 x 2 x 1 cm^3 size) according to ASTM D3171 was subjected to a compression test. The test was performed using the Instron Universal Testing Machine at room temperature (cross speed of 0.05 cm/min and full scale of 5000 kgf).

The Archimedes principle measured the density of casted glass with water as the immersion fluid. The measurements were performed at room temperature. All the weight measurements have been made using a digital balance (Sartorius, Model: BP221S, USA) having an accuracy of ± 0.0001 g. Density (ρ) of the sample was obtained by employing the relation as given below in equation--------- (3).

$$\text{Density} = Ma/(Ma-Mi) \times \text{Density of water} \quad \text{------} \quad (3)$$

Where, Ma (weight of sample in air), Mi (weight of sample in water) glass samples and 0.988 is the density of water at room temperature

2.9 Surface morphology of bioactive glass sample by SEM

0.2 gm of the powdered glass sample was soaked in 20 ml of SBF solution at 37 °C for different days, and the pH was measured using the microprocessor-based pH-EC meter (model-1611, ESICO-USA). The instrument was calibrated each time with standard buffer solutions of pH 4.00, and 7.00 at room temperature and pH values were measured at different time periods at a fixed interval. The glass powders (2 g) were pressed (load of 10 MPa) into pellet form using an evocable die to produce discs of 10 mm in dia for SEM analysis of bioactive glass samples. The pellets were immersed in SBF (20 ml) for 15 days at 37^oC, and the surface morphology of samples were analyzed before and after SBF treatment using a scanning electron microscope (SEM - Inspect S50, FEI). The samples were coated with gold (Au) by sputter coating instrument before analyzing by SEM.

2.10 Elastic properties of bioactive glasses

The ultrasonic wave velocities (longitudinal and shear) for cobalt oxide doped bioactive glass and base glass were measured using the Olympus instrument (M-45, USA) made by the USA. Bioactive glass samples were cut and polished in cubic pieces, and the couplant glycerin was used for finding longitudinal velocities and sonfech shear gel for the shear velocities of bioactive glass and its ceramic derivative. Using the formula the Young's modulus of elasticity, shear modulus of elasticity, the bulk modulus of elasticity and Poisson's ratio were found.

Formula of Young's Modulus of Elasticity, Shear Modulus of Elasticity, bulk modulus of Elasticity and Poisson's Ratio

Young's modulus (E) = $\rho V_L^2 [(1+d)(1-2d)/(1-d)]$,d= Poisson's Ratio

V_L= longitudinal velocity, V_T= Shear velocity

Shear Modulus (G) = $V_T^2 \rho$, ρ= density

Bulk modulus (K) = E/3(1-2d) , E= Young's modulus

Poisson's Ratio =$[1-2(V_T/V_L)^2] / [2-2(V_T/V_L)^2]$

Figure 2.5- Olympus instrument (M-45, USA) made by USA