Material and Methods

2.1. Preparation of Bioglass

Bioglass (45S5) with the chemical compositions 45%SiO_2, 24.5%CaO, 24.5%Na_2O and 6%P_2O_5 in (wt%) was prepared from reagent grade chemicals. Chemical were used fine-grained quartz (Merck, India) for silica. Lime and soda were introduced in the form of their respective anhydrous carbonates (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. Chemicals were weight, mixed and melted in 100 ml platinum crucible at 1400± 5˚C with air as furnace atmosphere for 4 hours. Melted glasses were poured into a mold to form in the desired shape, and it was milled to a powder form in a porcelain ball mill for 24 h in Fig. 2.1.

Figure 2.1: Melting of bioglass.

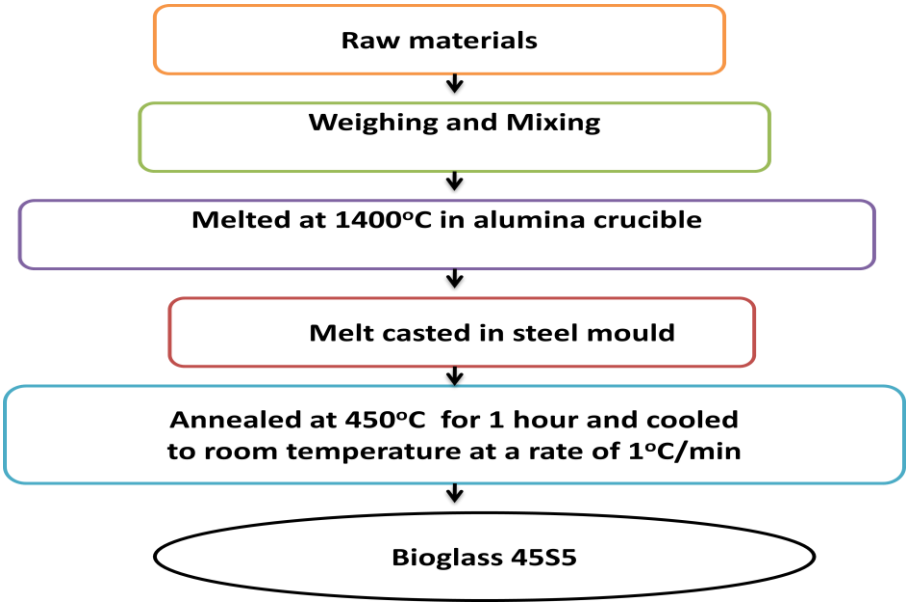

Figure 2.2: Flow chart preparation of bioglass.

2.2. Production of HA

In the present study, calcium nitrate tetrahydrate ($Ca(NO_3)_2.4H_2O$) (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 is given in Fig. 2.3.

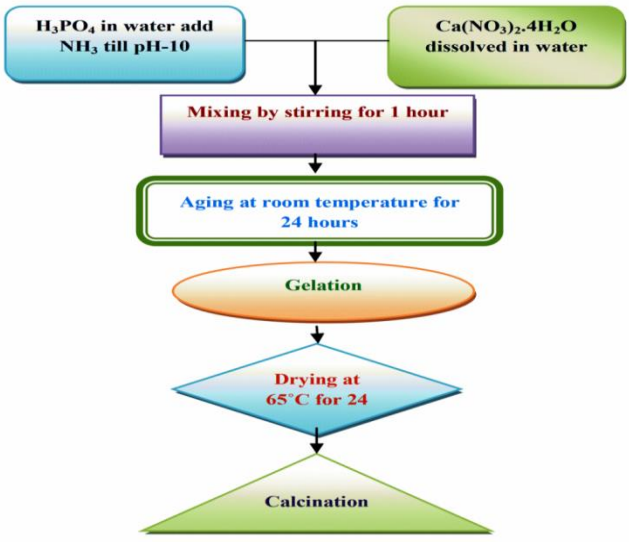

Figure 2.3: Flow chart preparation of hydroxyapatite.

2.3. Preparation of biocomposites

The HA, HA+TiO_2, HA+ZrO_2, HA+Fe_2O_3+CoO, HA+TiO_2+Nb_2O_5 powder were mixed each in proportional weight ratios with bioglass (45S5) powder, compacted at 1500 MPa pressure into cylindrical, rectangular die size and pellets samples were sintered at 1000, 1100°C to prepare the composites similarly as shown in Table 2.1.

Table 2.1: Composition of Bioglass and Biocomposite (BC1, BC2, BC3, BC4).

	Composition (wt %)			
BG (45S5)	45 SiO_2	24.5 Na_2O	24.5 CaO	6 P_2O_5
Biocomposite	**BG**		**HA**	
BC1	95		5	
BC2	90		10	
BC3	85		15	
BC4	80		20	
Biocomposite	**BG**	**HA**		**TiO2**
BGHATi1	90	5		5
BGHATi1	80	10		10
BGHATi1	70	15		15
BGHATi1	60	20		20
Biocomposite	**BG**	**HA**		**ZrO_2**
BHZ1	85	10		5
BHZ2	70	20		10
BHZ3	55	30		15
BHZ4	40	40		20
Biocomposite	**BG**	**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	10.0	2.5
Biocomposite	**BG**	**HA**	**TiO_2**	**Nb_2O_5**
BGHATiNb1	45	5	45	5
BGHATiNb2	40	10	40	10
BGHATiNb3	30	20	30	20
BGHATiNb4	25	25	25	25

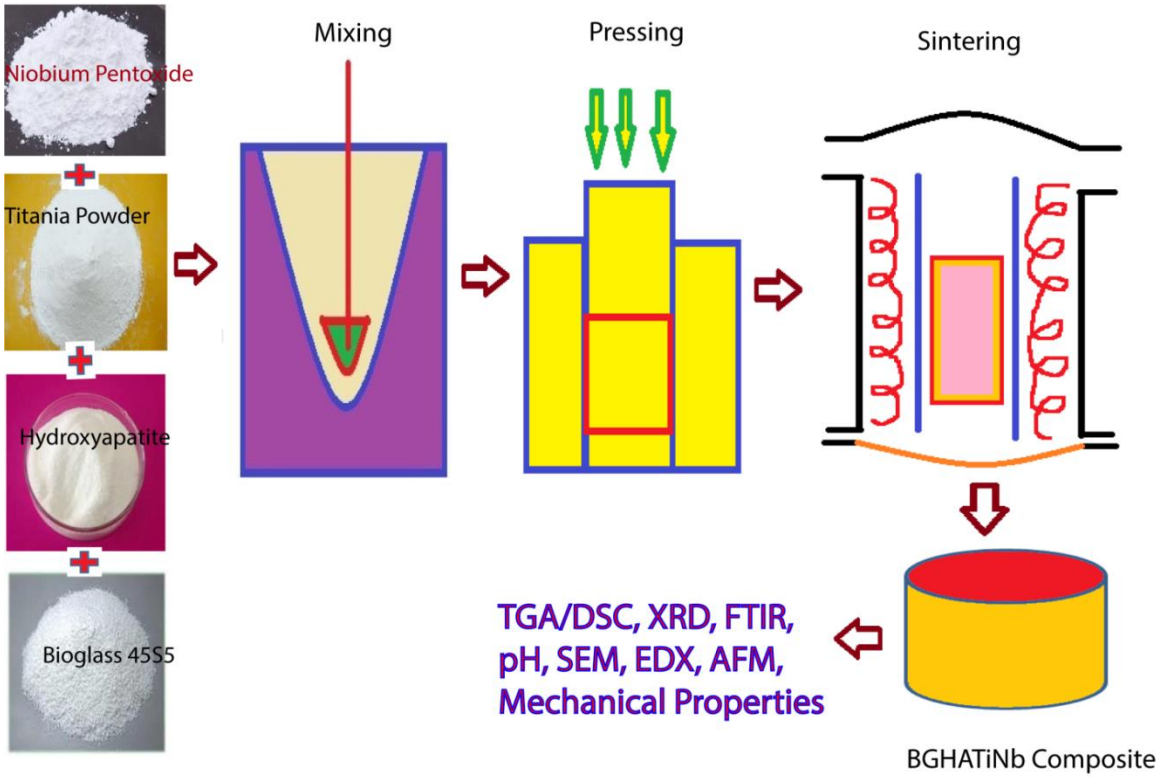

Figure 2.4: Preparation of biocomposite by powder metallurgy method.

2.4. Thermal Analysis

DSC (SETARAM Instrument, France) was carried out on powdered biocomposite samples in the air up to 900°C using powdered alumina as reference material at 10°C per min heating rate. The biocomposite nucleation and crystallization temperatures were carried out by DSC at higher crystallinity. When the sample was subjected to a controlled temperature condition, The TGA was used to measure the mass of a substance as a function of temperature.

Figure 2.5: DSC, TGA instrument.

2.5. X-ray diffraction analysis

Philips diffractometer (X'Pert MPD model) operating at 40 kV, 40 mA, and Cu-Kα radiation was used to identify the crystalline phases in the powder. The scanning rate was 0.058/min at 1s time step and analyses were performed within a range of 20-80°.

Figure 2.6: X-ray diffraction instrument.

2.6. Preparation of SBF

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

Table 2.2: The ions concentration of SBF solution and human blood plasma (mM/litre) [A. Agarwal et al.1997].

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

2.7. Fourier transform infrared (FTIR) spectroscopy

Evaluation of the attachment of silane to the biocomposite surface was done by Fourier transform infrared (FTIR) spectroscopy. Vertex 70 FTIR spectrophotometer (Bruker Optics, Ettlingen, Germany) were used to performed FTIR measurements. Biocomposite powder was dispensed over a diamond crystal of Attenuate Total Reflectance (ATR) accessory. A total of 16 scans were collected from 4000 cm^{-1} to 400 cm^{-1} at a 4 cm^{-1} resolution.

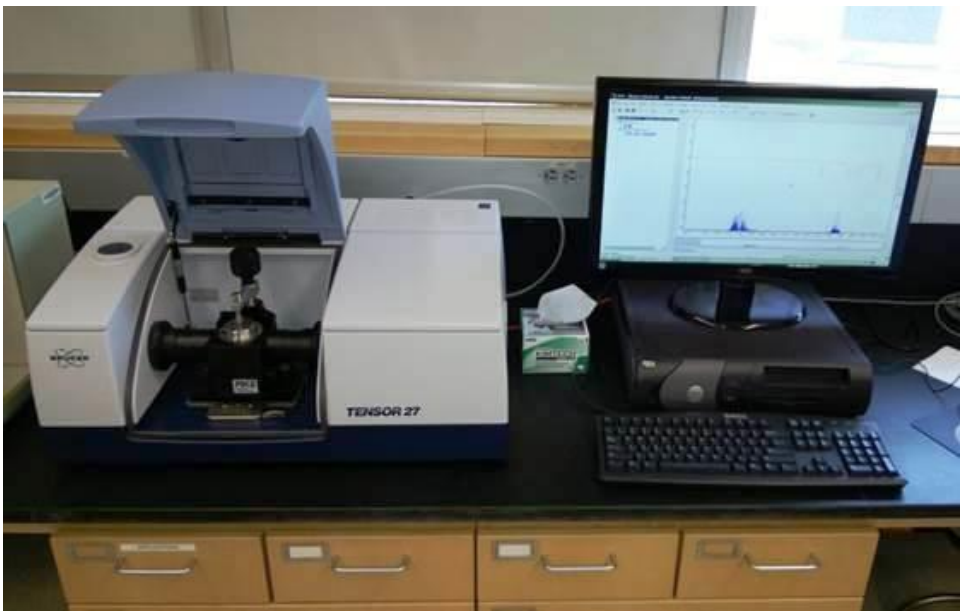

Figure 2.7: Fourier transform infrared (FTIR) spectroscopy.

2.8. pH Measurement

Bone-bonding ability of a material is often examined by immersion in simulated body fluid (SBF) which is an acellular fluid with ionic concentration nearly equal to those of human blood plasma. It has been shown that formation of hydroxycarbonate apatite (HCA) on materials surface in SBF is indicative of its bioactivity and is the first step for bonding to living bone *in vivo*.

Figure 2.8: pH meter.

2.9. Surface morphology of biocomposite sample by SEM and EDS

The evaluation morphology of the biocomposite samples was done by scanning electron microscopy (SEM) using Hitachi (TM3000 model) equipment. Energy dispersive spectrometry (EDS; Oxford ISIS 310, England) was used to determine the elemental composition and distribution for each specimen, utilizing an accelerating voltage of 15 kV, the working distance of 9 to 10 mm and electron voltage of 137 eV for the EDS detector.

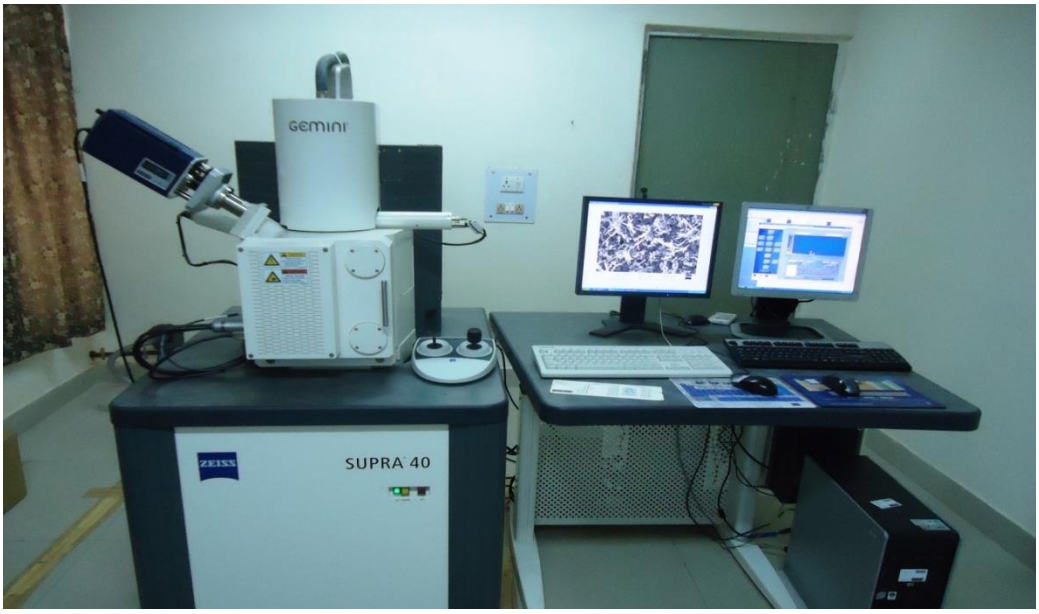

Figure 2.9: SEM and EDS instrument.

2.10. Physical and Mechanical Properties measurement

The density of biocomposite samples was determined by the Archimedes principle using a digital balance (Sartorius, model: BP221S, USA). The density of the sample was obtained by employing the equation (2.1).

$$\rho = \frac{Wa}{Wa-Wb}\rho b \qquad \text{------- (2.1)}$$

Figure 2.10: Density measurement instrument.

And the compressive strength of biocomposite samples was measured by a UTM (Shimadzu, Japan). The solid biocomposite samples were cubical (1cm×1cm×1cm). Using UTM, the samples were subjected to the compression load, and the test was performed at room temperature (cross speed 5mm/min). Four samples of each group were used for each point. The compressive strength was calculated using the following formulae.

$$\text{Compressive strength } (\sigma_C) = F/A \qquad \text{------- (2.2)}$$

F(N) is maximum compressive load during the test, and A is the initial area of the sample(cm^2).

Figure 2.11: UTM machine.

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