3.1 Introduction

The present chapter is a detailed discussed the synthesis method of metal alloy, bioactive glasses and their composites. In this chapter, the chemicals used for the synthesis of above mention also discussed and tabulated in table form. Works on the synthesis of composite also were showed as a flowchart diagram. Finally, the characterizations techniques used in the research work also discussed.

3.2 Materials

For the synthesis purpose, all materials are used as an analytical grade, and it is not further purified.

3.2.1 Material for the synthesis of Titanium based alloys

For the synthesis of titanium based alloys, the requirement of materials, their purity and manufacturing company name were listed in table 3.1 The metal powder a porous, brittle form of titanium, a highly ductile metal which has a high strength-to-weight ratio. The powder needs to be relatively fine. This ensures a high packing density, thereby decreasing porosity. This also assists in developing strong bonds during sintering, thereby increasing strength. The finer the powder the higher the final density and the stronger the component.

Chemical	Purity (%)	Manufacturing Company
Titanium Metal Powder	99%	LobaChemie
Silicon Metal Powder	99%	LobaChemie
Manganese Metal Powder	98%	LobaChemie
Niobium Metal Powder	95%	LobaChemie
Copper Metal Powder	98%	LobaChemie
Iron Metal Powder	97%	LobaChemie

Table 3.1 Required Chemical for synthesis of alloy matrix

3.2.2 Material for the synthesis of bioactive glasses

For the synthesis of bioactive glasses, the requirement of materials, their purity and manufacturing company name were listed in table 3.2

Chemical	Purity	Manufacturing Company
Quartz	99%	LobaChemie
Magnesium Oxide	99%	LobaChemie
Calcium Carbonate	99%	LobaChemie
Sodium Carbonate	99%	LobaChemie
Potassium carbonates	99%	LobaChemie
Boric acid	99%	LobaChemie
Di Sodium Hydrogen Phosphate	99%	LobaChemie

Table 3.2 list of chemical for glass preparation

3.2.4 Apparatus used in the synthesis of Alloy, glass and composite

3.2.4.1 High Energy Planetary Ball Mill-

The ball mill having a cylindrical container is used to ground the material .the cylindrical vessel which is partially filled with the material to be ground and grinding medium were also added .The steel balls are used as grinding media to prepare the powder form. The process of milling was done such that after each 15 min of cycle machine was stopped for 15 min.

Like in a planetary system the grinding jar rotates on a orbit around the centre. This rotational movement is the self-rotation of the grinding container superimposed. The resulting centrifugal and acting acceleration forces lead to strong grinding effects. Furthermore there are forces working according to the coriolis acceleration. The result is an intensive grinding effect between the grinding balls and the sample.

Depending on the speed ratio different movement patters of the grinding balls / media can be achieved. It can be achieved that the grinding media are crossing the grinding jar and loosen from the wall. At hitting the wall of the grinding jar the sample will be stressed. At a different motion pattern the grinding balls roll over the sample and stress the ground material [1].

If the size of the balls is too small, impact energy may be too low for alloying to take place. In order to increase impact energy without increasing the rotational speed, balls with high density such as tungsten balls may be employed [2].

Fig 3.1 planetary ball mill

3.2.4.2 Atmosphere controlled high temperature furnace-

To control and cease the oxidation of metal used in alloy and composite, we need to avoid the oxygen environment which is executed by use of controlled atmospheric furnace show below in fig 3.2. It consists of cylindrical tube to hold the sample in heating zone. The outer ends of the tubes are filled with sealing kit for atmosphere control. The water keeps circulating on the outer end of these tubes to keep the temperature down to avoid melting of sealing kit.

Fig 3.2 Atmosphere controlled high temperature furnace.

3.2.4.3 Uniaxial Pressing Machine-

Uniaxial pressing involves the compaction of powder into a rigid die by applying pressure in a single axial direction through a rigid punch or piston. The presses are usually mechanical or hydraulic and the pressing cycle repeats 6 to 100 times/min.

Fig 3.3 Uniaxial Pressing Machine.

3.2.5 Mechanical alloying

For the alloy mixing, we used the powder metallurgical approach bottom to top approach i.e. mechanical alloying. Titanium (Ti) metal powder is mechanical alloyed (MA) with different percentages of other metal powders in a high energy ball mill containing stainless steel vial along with tungsten balls for the synthesis of titanium based alloy powder at room temperature. The powder-to-ball weight ratio was maintained at 10:1. The vial of the mill was loaded with powder in an argon-filled glove-box to prevent oxidation.

The mill was run up to several hours for powder alloy preparation. Because of the ductility of metal powders; the milling is carefully controlled by adding a small amount of process control agent, (PCA), i.e., two weight % ethanol to the milling process was added. The PCA enhance milling efficiency by refreshing metal surface contacts. It also reduces the welding of the powder particles to each other and prevents the oxidation and contamination of material [3].

3.2.6 Glass Preparation

The glass batch containing required composition as weight percentage were prepared in different systems taking the starting raw material as Quartz(99% SiO_2), Magnesium Oxide (99%MgO) Calcium Carbonate (99%$CaCO_3$), Sodium Carbonate (99%Na_2CO_3), Potassium carbonate (98%K_2CO_3) and Boric acid (99%B_2O_3) . The raw materials were mixed for 30 minutes in a mortar pestle. These reagents were then melted in a 100 ml platinum-2% rhodium crucible for 4 hour kept in a global electric furnace at different temperature depending on composition in air atmosphere. The temperature of the furnace was controlled within ± 10°C by an automatic temperature indicator-cum-controller further, the glass melt was water quenched. Resultant glass was crushed and ball milled in the pot mill to bring in the powder form.

3.3.7 Composite Preparation

The mechanically alloyed metal powder matrix was reinforced with 0, 5, 10 and 20 percentage of melt-quenched bioactive glass and ball milled for 20 minutes for homogeneous mixing, and 0.3 % of carboxyl methyl cellulose (CMC) is added as an organic binder. A uniaxial cold press machine is used to consolidate the composites under the pressure of 450 MPa in the form of bars and pallets. The green compacts were pre-sintered first at 700^0C for 1 h for binder removal and then sintered in a high vacuum tube furnace with the holding time of 4 h at 1250 °C with the heating rate of 10 °C/min. The cooling is achieved first at 10 °C/min till 900°C and then allowed to cool at a faster rate of 40°C/min.

3.4. Characterization Techniques

3.4.1 Structural Characterization

X-ray diffraction is a primary non-destructive analytical technique for the determination of the chemical composition and crystallographic structure of the materials. It is also helpful to determine the lattice parameters, lattice defects, lattice strain, crystallite size, and phage of known and unknown materials. It is an electromagnetic wave with a wavelength of the order of one angstrom, and when it is incident upon the sample, diffraction from different atoms takes place. In the crystal, the arrangement of atoms are in a periodic manner, and diffracted x-rays from these atoms take to place an interfere [4].

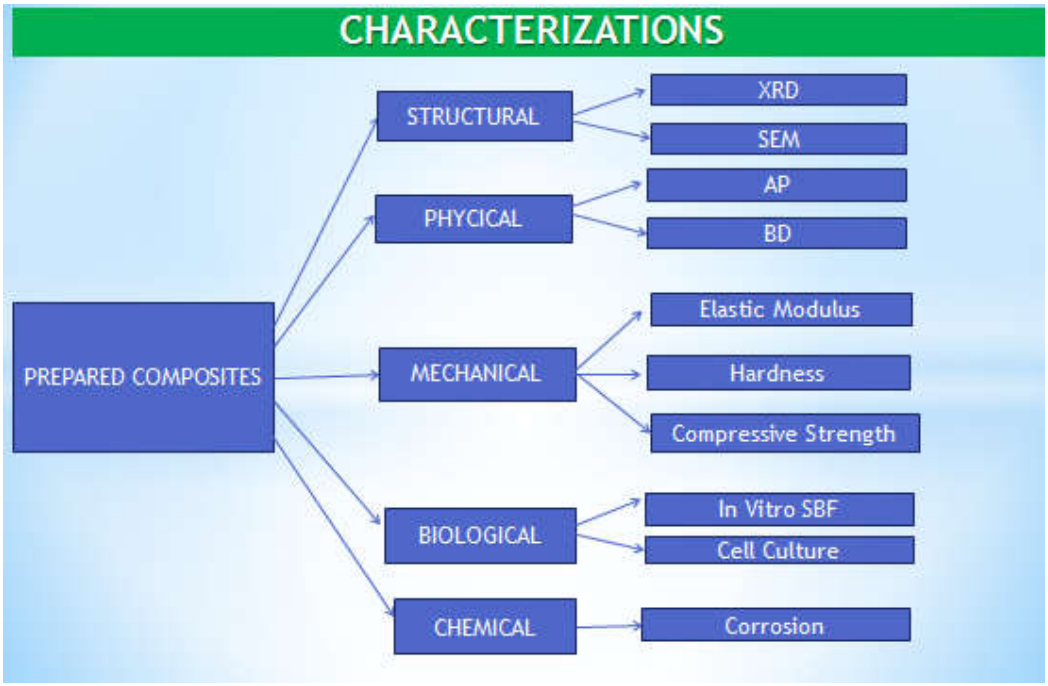

Fig.3.4 flow chart of characterized techniques used

The present atoms in the crystal formed sharp interference maxima (peaks) with the diffracted waves. This is directly related to the atomic distances. Therefore, the crystal structure of materials can be determined by measuring the distribution of the diffraction pattern. The inter-planer distance can be calculated according to Bragg's law as given below

$$2d\sin\theta = n\lambda \quad \text{---------- (3.6)}$$

Where 'θ' is the incident angle, 'λ' is the wavelength of the x-ray, and 'n' is an integer representing the order of the diffraction. This process is shown schematically in figure 3.9, and the experimental setup shown in figure 3.10 [5].

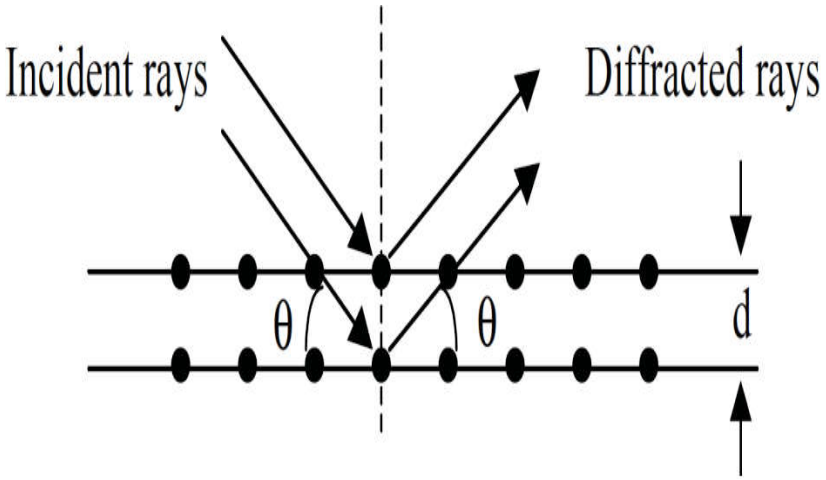

Figure. 3.5 Schematic of diffraction of X-rays by a crystal

Major phases in the composites were identified by compact powder XRD machine (Rigaku, Japan) using copper K_α radiation. Phase identification was completed by comparing the respective powder XRD patterns with the standard JCPDS database (PDF-2 Database 2003).

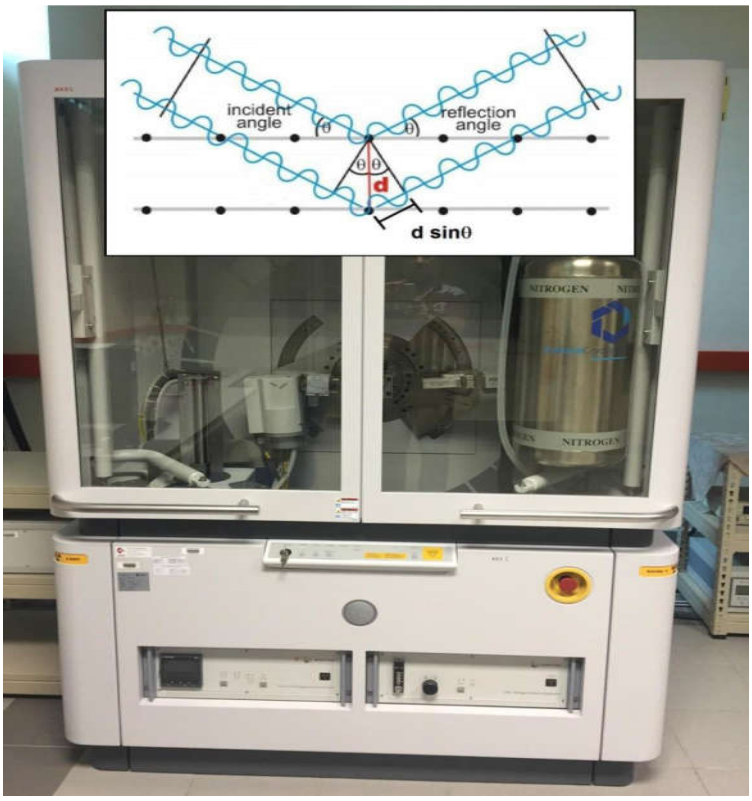

Figure 3.6 X-ray measurement setup

3.4.2 Scanning Electron Microscopy (SEM)

Scanning electron microscope (SEM), were used for examination of morphology, size, and shape, the elemental composition of the materials. In SEM the electron was emitted from a tungsten cathode. The emitted electron was focused into a very narrow intense beam through two successive condenser lenses. Finally, the narrow beam of the electron was further focused on the sample surface with the help of two pairs of coils. In this primary electrons were emitted and transmit their energy inelasticity to the atomic electrons of the crystal lattice. On the sample, the several scattering processes were taking place. Among them, some electrons managed to leaves the surface and collected by the detector and known as secondary electrons. Finally, the signal was amplified by the photomultiplier tube (PMT) and modulating this signal with the

intensity of a cathode ray tube (CRT), and the image of the sample was produced [6]. The good quality image of the sample was produced with a resolution of ~50A^0.

For microstructure determination, all samples were polished by emery papers of grades 1/0, 2/0, and 3/0 and then polish with the diamond paste of grade 1/4-OS-475. Samples were chemically etched with Kroll solution: 3 ml HF, 6 ml HNO_3, 100 ml H_2O and Scanning Electron Micrograph (SEM) were carried out using INSPECT 50 FEI instrument [7].

Archimedes method is used for the density measurement as it is more reliable (ASTM C373-88) and simple. The samples were immersed in water with the help of steel wire. Following mathematical expressions were used for the calculation of Bulk density and apparent porosity [8].

Apparent porosity (A.P %) = $\frac{W-D}{W-S}$ X 100, Where W=suspended weight in gm

Bulk Density (B.D gm/cm3) = $\frac{D}{W-S}$ S= Soaked weight in gm

D=Dry weight in gm

All the samples were immersed in SBF for bioactive analysis. The SBF was prepared according to Kokubo et al. [9] method which contains the same inorganic ion concentrations as presents in human body fluid. The preparation of SBF was carried out at 37°C by using all analytical grade reagent NaCl, KCl, $NaHCO_3$, $MgCl_2 \cdot 6H_2O$, $CaCl_2$ and KH_2PO_4 in distilled water. TRIS (tris-hydroxy methyl amino methane) and 1N HCl are used as a buffering agent to kept pH value at 7.2. In the sterilized container, the composites were immersed in SBF and incubated for seven days at 37 °C. The pH of SBF was measured regularly for seven days using a universal Bio-microprocessor pH

meter. The samples were further dried at 100 °C for 1 h and were subject to SEM (Inspect S50, FEI) for surface morphology.

3.4.3 Mechanical Testing

Compressive strength measurements were done according to ASTM C78M using a universal testing machine at a strain rate of 2 mm/min at room temperature [10]. Elastic moduli were evaluated by ultrasonic measurement gauge (45MG, Olympus, USA), by measuring density, velocities of the longitudinal and transversal wave are measured by ultrasonic pulse-echo technique. These measurements were taken with the help of the piezoelectric transducer (10 MHz), which makes contact with the sample via coupling gel. Vickers's hardness of the samples was evaluated by the indentation method with the help of Vickers's hardness tester (RVM 50) under the load of 1 kgf, and indentation is seen under an optical microscope. For all mechanical testing, four samples for each group are tested, and the mean and standard deviation were calculated. The results were shown as a mean value ± SD with the corresponding error bars.

3.4.4 Cytotoxicity assay

The lytic activity of the free compound S1, S2, S3, and S4 against the U2-OS cells was measured by cytotoxicity assay (CytoTox 96 cytotoxicity assay kit, Promega, USA) [11]. Tumor target cells (5×10^3) were co-cultured in the presence of increasing concentrations of the indicated formulations in a 96 well plate. The cells were incubated for 18 hours in a CO_2 incubator (37^0C, 5% CO_2). Percentage of cytotoxicity was ascertained from the undermentioned formula:

$$\%\ Cytotoxicity = \frac{(Experimental - Effector\ Spontaneous - Target\ Spontaneous)}{(Target\ Maximum - Target\ Spontaneous)} \times 100$$

3.4.5 Cell growth inhibition assay

MTT assay was performed to analyze growth inhibitory potential for the samples S1, S2, S3 or S4 against the U2-OS. The treatment of tumor target cells (5×10^3 cells /well) has been carried out with serial concentrations of the compounds in a 96 well culture plate and incubated for 48 hours at 37^0C, 5% CO_2. The proliferation of the tumor cells was also observed by MTT assay using Cell Titer 96 kit (Promega, USA). The absorbance (OD values) is measured at 570 nm in a micro plate reader (BioTek, USA) [12]. The undermentioned formula has been used for the calculation of percent inhibition of the tumor cells:

$$\% \text{ Growth Inhibition} = [1 - \frac{\text{Experimental } OD_{570}}{\text{Target } OD_{570}}] \times 100$$

The Experimental OD indicate the values of the tumor cells in the presence of the compounds S1-S4 whereas the Target OD represents the corresponding values of the tumor cells, grown alone in culture media[13].

The corrosion study has also been done with the help of weight loss method, where the composites were dipped into SBF solution at 37^0C for 42 days. Initially, the weight (dry weight) of all the samples was taken, and after immersion, the samples were taken out of SBF one by one at a time after 24, 48, 72, 96, 120, 144, 192, 216 and 240 hours. They were washed with the tap water to remove any corrosion products, and the weights (wet weight) of the samples were measured at a defined interval. The corrosion rate is calculated with the help of following mathematical expression [14].

$$\text{Corrosion Rate in mili-meter per year} = 87.6 \frac{W}{DAT}$$

Where A= Area of samples in cm^2

D= Density of the sample in g/cm^3.

The w=weight loss in mg

T=Time of immersion in hours

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