CHAPTER-7

Summary and future work

Summary

This thesis deals with three types of bioactive glasses.

The first one is doping titanium dioxide for SiO_2 in 1393 bioactive glass,

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

The second one is doping zirconium dioxide for SiO_2 in 1393 bioactive glass,

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

The third one is doping cobalt oxide for SiO_2 in 1393 bioactive glass.

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

The fourth one is doping zirconium dioxide for CaO in 1393 bioactive .

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

CoO, TiO_2, and ZrO_2 were added to the glass composition for silica and CaO in different concentrations to yield a charge-balanced series (CB) of bioactive glasses. The other components of the bioactive glass were kept constant.

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.

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 + $4NH_3$ + $3H_2O$

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).

After the preparation of sample and SBF the following characterization were done foe samples:

Powder X-ray diffraction (XRD) measurements,

Structural analysis of bioactive glass by FTIR spectrometry,

In vitro bioactivity study of bioactive glass, mechanical behavior measurements,

pH measurement,

Surface morphology of bioactive glass sample by SEM

Elastic properties of bioactive glasses.

The following results were observed after the comparative analysis of samples before and after immersion in SBF-

It is concluded that an increase in titanium oxide content in this series of glasses resulted in an increase in bioactivity. This is also supported by pH and SEM analysis. FTIR results showed the silicate network structure in prepared bioactive glass and increasing the titanium oxide content in 1393 bioactive glass increase the density, flexural strength, compressive strength, and microhardness.

Physicomechanical and bioactive properties of zirconium dioxide substituted 1393 bioactive glasses were analysed. The following conclusions were drawn from this investigation. The XRD analysis of the bioactive glass before immersing into SBF showed the amorphous nature of the glass. FTIR reflectance spectra, pH behavior, XRD and SEM images indicate the formation of hydroxylapatite (HA) layer on the surface of the zirconium dioxide containing bioactive glasses after immersing in simulated body fluid (SBF). FTIR results showed the silicate network structure in prepared bioactive glass and increasing the zirconium dioxide content in 1393 bioactive glass increase the density, flexural strength, compressive strength, and microhardness. Hence, the present investigation clearly indicates that ZrO_2 substituted bioactive glass would be potential biomaterials for biomedical applications.

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.

In the analysis of ZrO_2 doped for CaO samples the XRD analysis showed the amorphous nature of the glass and FTIR absorbance spectra, pH behavior; XRD and SEM images show the formation of HCA layer on the surface bioactive glasses after putting in SBF. Densities of substituted bioactive glasses are increased with increasing concentration of ZrO_2 while their Chemical durability decreased.

So it can be concluded from the experimental work that all the ZrO_2 substituted bioactive glass have shown improved properties. Among all the samples, the G-4 glass is the best one as it has shown high pH value which suggests the formation of HCA layer. It can be observed from the FTIR diagram of G-3 that all the bonds are showing prominent peaks and SEM images are also showing impressive results of HA layer formation. The prepared bioactive glasses can be used for bone tissue engineering applications.

The following conclusions are obtained from this investigation.

- The formation of hydroxyl carbonate apatite (HCA) layer on the surface of the bioactive glass samples were confirmed by XRD, FTIR, pH and SEM after immersing in SBF solution.
- Density, flexural strength, compressive strength, micro hardness and elastic moduli of bioactive glasses increased with increasing CoO, TiO_2 and ZrO_2 concentration in base bioactive glasses.
- Elastic moduli of bioactive glasses increased with increasing CoO, TiO_2 and ZrO_2 concentration in 1393 bioactive glasses while the Poisson's ratio slightly decreased.

Overall conclusion of present investigation is that bioactive glasses obtained by substitution of 1% by weight of CoO, TiO_2 and ZrO_2 in base bioactive glasses may be more beneficial as a bioactive material.

Future work

- Cell culture studies like In vitro cytotoxicity, cell morphology, cell viability and proliferation.
- Metabolic activity estimation using MTT assay
- Bioactivity evaluation by ALP activity
- Biomineralization by Alizarin Red S assay
- Osteogenic potential estimation by Immunocytochemistry for Osteocalcin and Osterix
- Vascularization or angiogenesis (formation of blood vessels), Osteogenesis (formation of bone tissue)
- Antibacterial study: using gram+ and gram- bacteria
- In-vivo Animal study:
- The in-vivo study followed by histopathological analysis will confirm the suitability of developed scaffold for neo-bone tissue regeneration.