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In Vitro Evaluation of Silicate Based Bioactive Glass

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Abstract: In the present paper, borosilicate bioactive glasses with composition $\text{Na}_2\text{O-Li}_2\text{O-SrO-CdO-B}_2\text{O}_3\text{-SiO}_2\text{-P}_2\text{O}_5$ and $\text{Na}_2\text{O-LiBr-SrO-CdO-B}_2\text{O}_3\text{-SiO}_2\text{-P}_2\text{O}_5$ were prepared and their bioactivity is reported. The conventional melt quench method was used for glass synthesis. The synthesized glasses were characterized by using Scanning electron microscopy (SEM) and energy dispersive X-ray analysis (EDX). In Vitro surface reactivity of bioactive glasses was evaluated by examining the formation of hydroxyl carbonate apatite (HCA) layer on their surface after soaking in simulated body fluid (SBF). The change in pH of the simulated body fluid (SBF) solution was measured for different time intervals. The weight loss of the synthesized glass was observed after the immersion in simulated body fluid (SBF). The Scanning electron microscopy (SEM) and energy dispersive X-ray analysis (EDX) results confirmed the formation of hydroxyl apatite layer (HCA) on the surface of synthesized glass, hence the in vitro bioactivity nature of the synthesized glass.

Keywords: Bioactive Glass; pH changes; conventional method; simulated body fluid;

I. INTRODUCTION

Material sciences have experienced immense progress in the development of new materials in the past 30 years[1]. Developing new materials or modifying the existing materials composition to enhance the properties of existing materials for various applications is always a promising challenge in the field of material sciences, particularly in the development of biomaterials with new composition. Biomaterials are widely used in the form of implants like bone plates, joint replacements, dental implants, cavity fillers etc. and also as medical devices like artificial hearts, pacemakers etc[2],[3], [4].

The main requisite of biomaterial is its acceptability by the human body [5]. Biomaterials are broadly classified as bio inert, bioactive and bio restorable[6]. Most of the bioinert materials are metals made up of Al_2O_3 , ZrO_2 etc [7]. Bioinert materials bonding at the host tissue is very minimal almost inert[8],[9],[10].

After the spectacular invention of first bioactive glass by Hench et al. In 1969 at the University of Florida the field of bioactive materials came into focus then after numbers of researchers are focussing in this field to develop new bioactive materials with varying compositions to reduce the limitations of present bioactive materials[11],[12]. Bio resorbable materials are capable to dissolve completely with the host tissue in which it is incorporated [13]. Hench and his colleagues developed a novel, miracle bioactive material with glass composition $\text{Na}_2\text{O-CaO-P}_2\text{O}_5\text{-SiO}_2$ [14]. Bio active material may be synthetic but should chemically bond with surrounding body tissue when implanted into human body[15].

The response of the body at the interface of body tissue and bioactive implant expected to be nontoxic, stable and mechanically strong enough to withstand with load bearing applications[16],[17]. The first and foremost requirement of bioactive material is its compatibility [18]. This can be investigated by examining the formation of calcium phosphate rich layer on the surface of the bioactive glass when immersed in physiological body fluid[19]. The simulated body fluid (SBF) can be used as physiological body fluid which mimics the human blood plasma. The ion concentrations of SBF is nearly equal to human blood plasma. This was successfully explained by Kokubo et al. [19]. Simulated body fluid is useful to conduct in vitro studies of bioactivity[20]. When bioactive material is in contact with simulated body fluid a calcium phosphate layer is formed, then after nucleation and growth of an apatite like phase gives rise to crystalline hydroxyl carbonate apatite (HCA) layer formation on the glass surface[21], [22]. The formation of calcium apatite layer is the main requirement to examine the bioactivity nature of the bioactive material.

In the present study new bioactive active glass with composition $\text{Na}_2\text{O-Li}_2\text{O-SrO-CdO-B}_2\text{O}_3\text{-SiO}_2\text{-P}_2\text{O}_5$ and $\text{Na}_2\text{O-LiBr-SrO-CdO-B}_2\text{O}_3\text{-SiO}_2\text{-P}_2\text{O}_5$, which includes alkali halides was reported. The synthesized glasses were prepared by reliable and simple melt quench method. This method is suitable for mass production of glass. For in vitro studies the synthesized glasses were characterized by Scanning electron microscopy (SEM) and energy dispersive X-ray analysis (EDX).

II. MATERIALS AND EXPERIMENTAL PROCEDURES

A. preparation of glass

Borosilicate bioactive glass sample BG1 with composition $8\text{Na}_2\text{O}-8\text{Li}_2\text{O}-8\text{SrO}-20\text{CdO}-40\text{B}_2\text{O}_3-10\text{SiO}_2-6\text{P}_2\text{O}_5$ in wt% was prepared by using analytical grades of Na_2CO_3 , Li_2O , SrCO_3 , CdCO_3 , H_3BO_3 , SiO_2 and P_2O_5 . The phosphate glass sample BG2 with composition $8\text{Na}_2\text{O}-8\text{LiBr}-8\text{SrO}-20\text{CdO}-40\text{B}_2\text{O}_3-10\text{SiO}_2-6\text{P}_2\text{O}_5$ in wt% was prepared by using analytical grades of sodium carbonate Na_2CO_3 , lithium bromide LiBr , strontium carbonate SrCO_3 , CdCO_3 , H_3BO_3 , SiO_2 and P_2O_5 . The glass batch was melted at 1100°C using electrical muffle furnace. Preheated steel plate used for quenching.

Simulated body fluid was prepared according to the method explained by kokubo et al. Simulated body fluid ion concentrations are almost equal to human blood plasma [23]. The reagent grade chemicals sodium chloride NaCl , NaHCO_3 , potassium chloride KCl , $\text{K}_2\text{HPO}_4 \cdot 3\text{H}_2\text{O}$, $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$, CaCl_2 , Na_2SO_4 were mixed one after one in appropriate portions using magnetic stirrer in distilled water [24]. The solution is buffered at pH of 7.4 with suitable amount of tris - hydroxymethyl amino methane $(\text{CH}_2\text{OH})_3\text{CNH}_2$ and hydrochloric acid HCl .

B. In vitro Bioactivity study

For in vitro bioactivity study the synthesized glass sample with composition $8\text{Na}_2\text{O}-8\text{Li}_2\text{O}-8\text{SrO}-20\text{CdO}-40\text{B}_2\text{O}_3-10\text{SiO}_2-6\text{P}_2\text{O}_5$ in wt% (BG1) was soaked in simulated body fluid (SBF) for 15 days. The synthesized glass sample with composition $8\text{Na}_2\text{O}-8\text{LiBr}-8\text{SrO}-20\text{CdO}-40\text{B}_2\text{O}_3-10\text{SiO}_2-6\text{P}_2\text{O}_5$ in wt% (BG2) was soaked in simulated body fluid (SBF) for 15 days. The temperature of the incubation apparatus was maintained at 36.5°C . The samples were taken into plastic containers and soaked in SBF and were placed in incubation chamber for 15 days. After 15 days the samples BG1 and BG2 were taken from the incubation apparatus and cleaned gently with ethanol and then with distilled water and were left to dry at room temperature in a desiccators. The dried samples BG1 and BG2 were characterized by using Scanning Electron Microscopy (SEM) and Energy Dispersive X-ray analyser (EDX). The weight loss of the synthesized glass was observed.

III. RESULTS AND DISCUSSION

The Scanning Electron Microscopy (SEM) was used for characterization of glass. The surface morphology and micro particles composition on the surface of the glass were studied using a (SEM/EDX) Zeiss ULTRA plus Scanning Electron Microscope (SEM) (Osmania University, Hyderabad) and energy dispersive X-ray analyser (EDX). The Energy Dispersive X-ray Spectroscopy (EDS) analysis at an accelerating voltage of 10kV was carried out to determine the presence of elements on the synthesized glass surface. The micrograph provides visual evidence of the formation of an apatite layer on the surface of a synthesized glass. Fig 1 Shows the SEM image of bioactive glass sample BG1 of composition $8\text{Na}_2\text{O}-8\text{Li}_2\text{O}-8\text{SrO}-20\text{CdO}-40\text{B}_2\text{O}_3-10\text{SiO}_2-6\text{P}_2\text{O}_5$ at $200\mu\text{m}$ magnification.

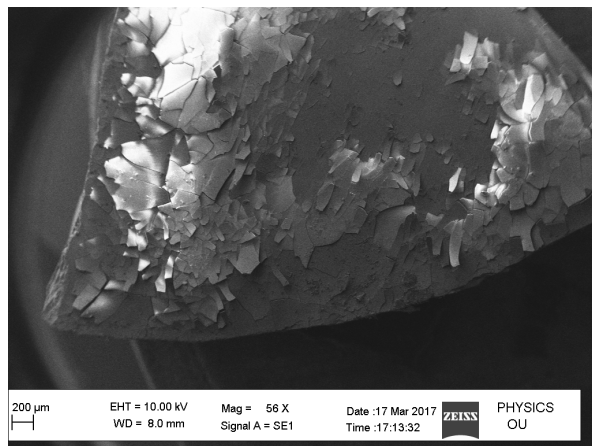


Fig.1 SEM image of synthesized glass BG1

Fig 2 and fig 3 shows the SEM micrograph of silicate based bioactive glass BG1 of composition $8\text{Na}_2\text{O}-8\text{Li}_2\text{O}-8\text{SrO}-20\text{CdO}-40\text{B}_2\text{O}_3-10\text{SiO}_2-6\text{P}_2\text{O}_5$ at $1\mu\text{m}$ and $2\mu\text{m}$ magnification. The result indicates the presence of apatite and even spherical shaped micro particles are observed on the surface of synthesized glass.

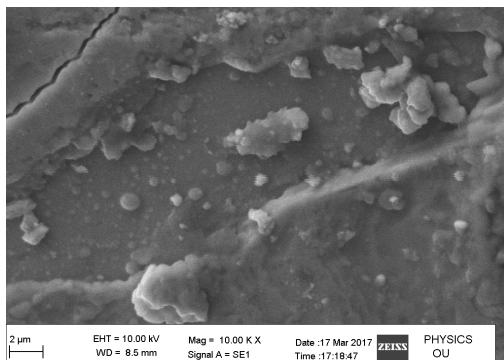


Fig.2 SEM image of synthesized glass BG1

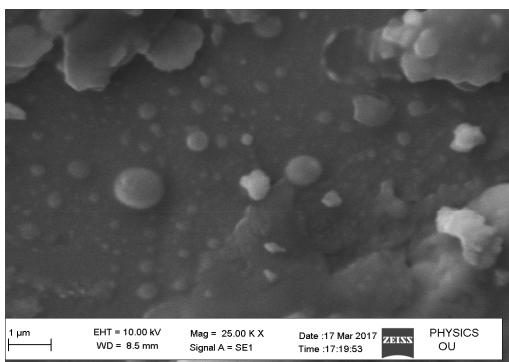


Fig.3 SEM image of synthesized glass BG1

Fig 4 and fig 5 Shows the SEM image of bioactive glass sample BG2 of composition $8\text{Na}_2\text{O}-8\text{LiBr}-8\text{SrO}-20\text{CdO}-40\text{B}_2\text{O}_3-10\text{SiO}_2-6\text{P}_2\text{O}_5$ at $2\mu\text{m}$ magnification after immersion of 15 days.

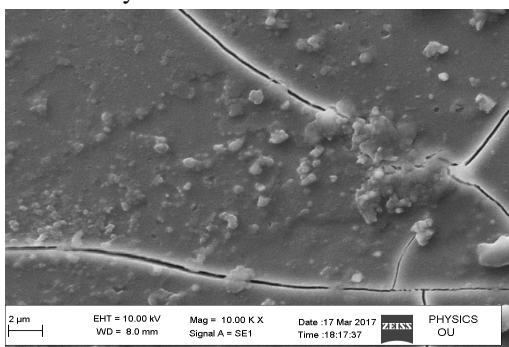


Fig.4 SEM image of synthesized glass BG2

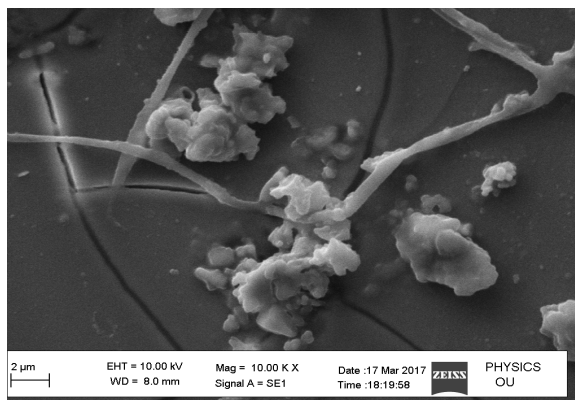


Fig.5 SEM image of synthesized glass BG2

The EDS analysis of synthesized glasses BG1 and BG2 reveals the formation of a hydroxyl apatite layer on the surface of BG1 and BG2 after immersion in SBF for 15 days. The precipitates on the surface of the sample are made up of calcium and phosphorous are shown in fig 8, fig 6 and fig 7. The presence of small quantities of P, Ca, Si, Mg are shown in EDS. It may be concluded that the surface layer forms crystalline phase of hydroxyl apatite layer.

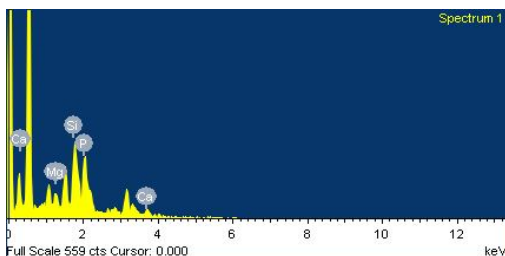


Fig. 6 EDX ata of synthesized glass BG1

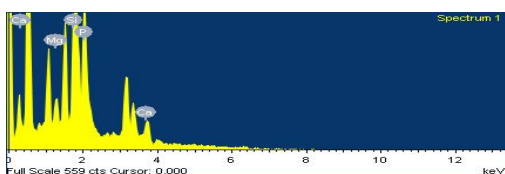


Fig. 7 EDX data of synthesized glass BG2

The weight loss of the synthesized glass BG1 and BG2 was observed by measuring initial weight of the sample and the final weight of the sample after soaking in simulated body fluid. The weight loss indicates the synthesized glass is biodegradable hence the prepared glass exhibits bioactivity. The glass samples were immersed in simulated body fluid and the changes in pH of simulated body fluid was determined with pH meter. The result indicated an increase in pH upon immersion in SBF is as shown in fig. 8. Initially the pH increased with increase in time for synthesized glass and then there was a slight reduction in pH. The variation of pH indicates that the exchange of ions takes place with the release of Si and Ca ions. Hence the leaching of soluble silica is an indication of bonding ability of the BG1 and BG2.

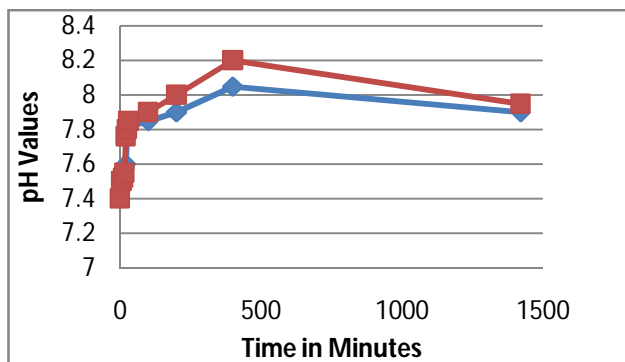


Fig. 8 pH changes in synthesized glass

- ◆ 8Na₂O-8Li₂O-8SrO-20CdO-40B₂O₃-10SiO₂-6P₂O₅
- 8Na₂O-8LiBr-8SrO-20CdO-40B₂O₃-10SiO₂-6P₂O₅

IV. CONCLUSIONS

In the present paper, the in vitro bioactivity of the synthesized glass BG1 and BG2 with composition 8Na₂O-8Li₂O-8SrO-20CdO-40B₂O₃-10SiO₂-6P₂O₅ and 8Na₂O-8LiBr-8SrO-20CdO-40B₂O₃-10SiO₂-6P₂O₅ were reported. The characterization results of SEM micrograph and EDS data revealed the formation of hydroxyl apatite layer on the surface of synthesized glass BG1 and BG2. The change in pH of the simulated body fluid and weight loss of the synthesized glass BG1 and BG2 shows the leaching of soluble silica hence the formation of silica based hydroxyl apatite layer on the surface of the synthesized glass.

V. ACKNOWLEDGMENT

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