https://dx.doi.org/10.22161/ijcmp.1.4.1 *ISSN: 2456-866X*

Synthesis and Characterization of Bioactive Glass - In Vitro Studies

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Abstract*—Melt quench experimental method was used to prepare a new B2O3 based bioactive glasses with low lithium bromide and low lithium fluoride content. The aim of this work is to investigate the bioactivity nature of prepared glass samples with composition Na_2O-LiBr-SrO-CdO-B_2O_3-P_2O_5 and Na_2O-LiF-SrO-CdO-B_2O_3-P_2O_5.The melt derived glass ceramics were characterized by using X-ray Diffraction (XRD), Scanning Electron Microscopy (SEM) and Energy Dispersive Spectroscopy (EDS). For In vitro studies, the bone bonding ability is evaluated by examining the formation of hydroxyl apatite layer (HCA) on its surface when treated in Simulated Body Fluid (SBF) for few days. SEM and EDS analysis were used for surface morphology of the glass ceramics as well as to detect the presence of crystalline phase hydroxyl carbonate apatite (HCA) formation onto the surface of glass ceramics. SEM and EDS confirms the in vitro bioactivity of glass ceramics.*

***Keywords*— Bioactivity, Bone bonding, Hydroxyl carbonate apatite, In Vitro studies, SBF.**

I. INTRODUCTION

A Biomaterial is a synthetic material capable to chemically bond with living tissue [1]. In 1986, at the Consensus Conference of the European Society for Biomaterials, defined the biomaterial as "a nonviable material used in a medical device, intended to interact with biological systems" [2]. The main purpose of biomaterials is to repair or replace the parts of the human body when used as body implants [3] [4]. Biomaterials are broadly classified as bio inert, bioactive and bio resorbable [5]. The first generation bio inert materials are biologically inert and are unable to bond to living tissues results revision surgeries in long term implant failures [6]. This difficulty was reduced after the discovery of second generation new miracle material by Hench in 1969 with composition 24.5Na_2O-24.5CaO-6P_2O_5-45SiO_2 in wt% called 45S5 by melt quench method [7]. Bio active materials are capable to bond chemically at the interface of the implant and body tissue. In the present work in vitro studies of bioactive materials were carried out. The prepared Simulated Body Fluid (SBF) was used for in vitro studies. When the bio active glasses are immersed in Simulated Body Fluid (SBF) there will be formation of calcium rich phosphate layer called hydroxyl carbonate apatite (HCA) on their surface[8] [9]. This is the main requisite to test the bioactivity of glass [10] [11]. In search of new bioactive glass compositions alkali halogens were included in the prepared glass samples. In the present work the bioactivity of new glass composition Na_2O-LiBr-SrO-CdO-B_2O_3-P_2O_5 and Na_2O-LiF-SrO-CdO-B_2O_3-P_2O_5 was reported. X-ray diffraction (XRD), Scanning Electron Microscopy (SEM) and Energy Dispersive Spectroscopy (EDS) techniques were performed to investigate the bioactivity of the prepared glass samples. The result confirms the formation of hydroxyl carbonate apatite (HCA) on the glass surface after immersion in Simulated Body Fluid (SBF) for 7 days at 37^0C. Bioactive materials are useful in medicine particularly for tissue engineering and dental treatment.

II. EXPERIMENTAL

The phosphate glass sample1 with composition 8Na_2O-8LiBr-8SrO-20CdO-50B_2O_3-6P_2O_5 in wt% was melted using analytical grades of Na_2CO_3, LiBr, $SrCO_3$, $CdCO_3$, H_3BO_3, and P_2O_5. The glass sample2 with composition 8Na2O-8LiF-8SrO-20CdO-50B_2O_3-6P_2O_5 in wt% was melted using analytical grades of Na_2CO_3, LiF, $SrCO_3$, $CdCO_3$, H_3BO_3, and P_2O_5. The glass was melted in porcelain crucible at 1100^0C using muffle furnace. After melting glass samples subjected to conventional quenching method.

For in vitro studies of glass simulated body fluid was prepared according to the method proposed by kokubo simply called kokubo's solution [12] [13]. Simulated body fluid (SBF) ion concentrations are nearly equal to human blood plasma. Hence simulated body fluid was used to test the in vitro bioactivity of prepared phosphate glass samples. The analytical grade chemicals NaCl, NaHCO3, KCl, K2HPO_4.3H_2O, $MgCl_2$.6H2O, $CaCl_2$, Na_2SO_4 were mixed in appropriate portions in an ion exchanged distilled

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water then it is buffered at pH of 7.4 with appropriate amount of tris-hydroxymethyl amino methane $(CH_2OH)_3CNH_2$ and HCl.

The prepared samples were soaked in simulated body fluid (SBF) for 7 days at 36.5^0C room temperature by using incubation chamber [14] [15] . The change in pH of the solution with time was recorded by using pH meter. After 7 days the samples were taken from the incubator and cleaned with ethanol and then with distilled water. Finally the samples were left to dry at ambient temperature in a dessicator [17]. The dried samples were analysed by using X-ray diffraction technique (XRD), Scanning Electron Microscopy (SEM) and Energy Dispersive X-ray Spectroscopy (EDS).

III. RESULTS

The XRD analysis was carried out on the obtained glass samples by using Philips X'pert diffractometer (Cu kα radiation) in the 2θ range 5-80 ^{0} with step size of 0.020 operating at 40kV, 30mA. X-ray diffraction (XRD) results for the glass sample1 $8Na_2O$-8LiBr-8SrO-20CdO-$50B_2O_3$-$6P_2O_5$ after soaking in simulated body fluid is as shown in Fig.1.The XRD pattern of the glass shows broad band characteristic of an amorphous material, crystallization peaks can be observed at $25 – 30^0$ range and $46 – 50^0$ range in the XRD pattern. The peaks can be attributed to the precipitation of hydroxyl carbonate apatite (HCA) on the glass surface.

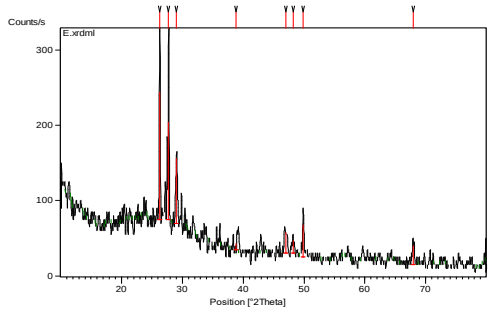

Fig. 1: XRD pattern of glass sample1

X-ray diffraction (XRD) result for glass sample2 $8Na_2O$-8LiF-8SrO-20CdO-$50B_2O_3$-$6P_2O_5$ is as shown in Fig.2. The XRD pattern of the glass shows broad band characteristic of an amorphous material, crystallization peaks can be observed at $30 – 35^0$ range and $46 – 48^0$ range in the XRD pattern. The peaks can be attributed to the precipitation of hydroxyl carbonate apatite (HCA) on the glass surface.

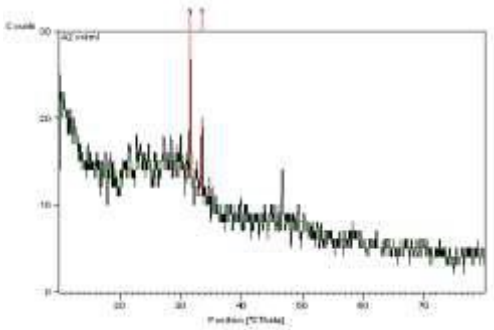

Fig. 2: XRD pattern of glass sample2

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) and energy dispersive X-ray analyser (EDX). The Energy Dispersive Spectroscopy (EDS) analysis at an accelerating voltage of 15kV was carried out to determine the presence of elements on the prepared glass sample surface. Fig 3 and Fig 4 shows the SEM micrograph of bioactive glass sample1 of composition $8Na_2O$-8LiBr-8SrO-20CdO-$50B_2O_3$-$6P_2O_5$. The SEM micrograph provides visual evidence of the formation of hydroxyl carbonate apatite (HCA) layer on the surface of glass sample1.

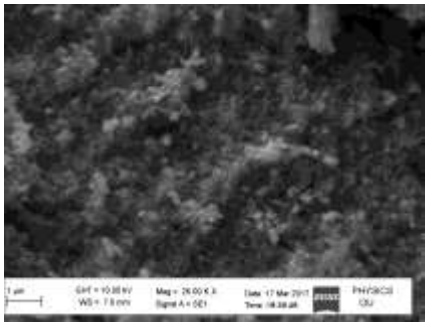

Fig. 3: SEM photograph of glass sample1

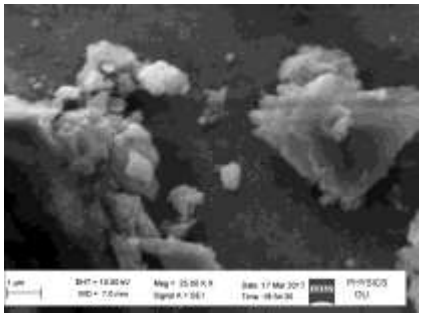

Fig. 4: SEM photograph of glass sample1

The EDS analysis of glass sample1 reveals the formation of HCA on the surface of glass after soaking in SBF for 7 days is as shown in Fig 5. The precipitates on the surface of the sample shows the presence of small quantities of Na, P, Ca, C, O and Sr. `

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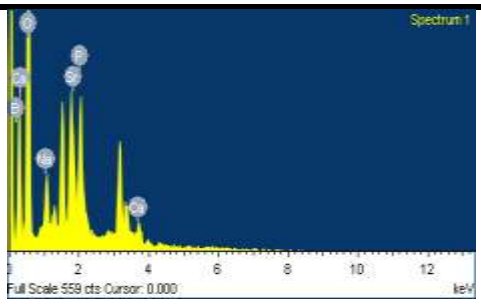

Fig. 5: EDS analysis of glass sample1

Fig 6 and Fig 7 presents the SEM image of bioactive glass sample2 of composition $8Na_2O$-8LiF-8SrO-20CdO-$50B_2O_3$-$6P_2O_5$. The SEM image provides visual evidence of the formation of hydroxyl carbonate apatite (HCA) layer on the surface of glass sample2.

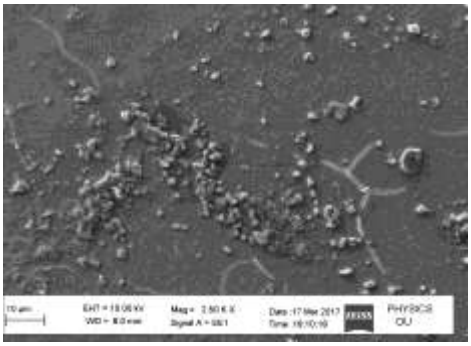

Fig. 6: SEM photograph of glass sample2

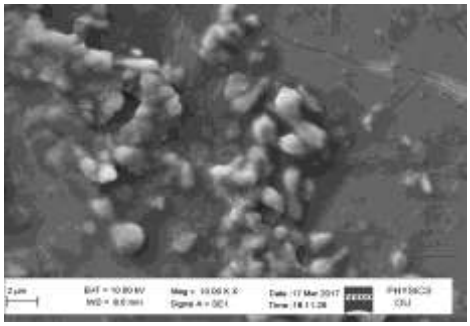

Fig. 7: SEM photograph of glass sample2

The EDS analysis of glass sample2 shows the formation of HCA on the surface of glass after soaking in SBF for 7 days is as shown in Fig 8. The precipitates on the surface of the sample show the presence of small quantities of Na, P, Ca, C, and Sr.

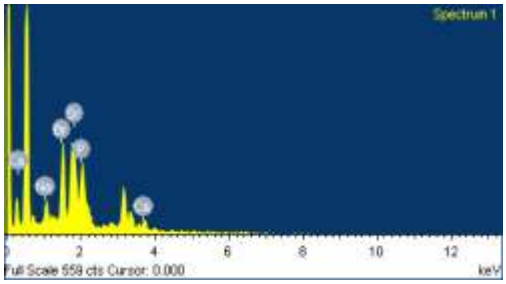

Fig. 8: EDS analysis of glass sample2

After immersion of glass samples in simulated body fluid (SBF) the changes in pH of immersion solution was determined with pH meter. Fig 9 presents the change in pH as a function of time. The pH values increased with time for glasses and there was a slight reduction in pH at 300 - 400 minutes in SBF.

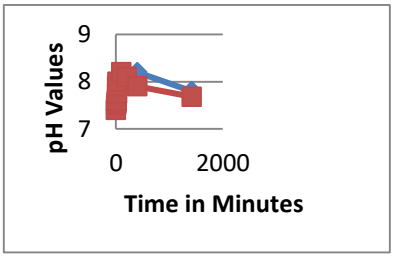

Fig. 9: changes in pH as a function of time

B *$8Na_2O$-8LiBr-8SrO-20CdO-$50B_2O_3$-$6P_2O_5$*
C *$8Na_2O$-8LiF-8SrO-20CdO-$50B_2O_3$-$6P_2O_5$*

IV. CONCLUSION

The present work reports the results of in vitro test of bioactivity of glass ceramics with new composition $8Na_2O$-8LiBr-8SrO-20CdO-$50B_2O_3$-$6P_2O_5$ and $8Na_2O$-8LiF-8SrO-20CdO-$50B_2O_3$-$6P_2O_5$ for the first time of our knowledge. The precipitation of hydroxyl carbonate layer on the surface glass ceramics confirms the prepared two glass samples are biocompatible. The in vitro reactivity of a bioactive glass can be predicted as a function of glass composition. The pH in immersion solution SBF increased to different levels depending on glass composition. Alkali halogens included in the glass composition shows the bio activity of glass samples. After in vivo test these glasses with optimized composition may be useful for tissue engineering and dental applications.

ACKNOWLEDGEMENTS

The authors are gratefully thankful to the Department of Physics, Osmania University, Hyderabad, Telangana, India for providing XRD, SEM, EDS results.

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