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In Vitro Bioactivity and Chemical Properties in TiO_2 Doped 45S5 Bioactive Glasses and Glass -Ceramics

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Abstract - Silica-based bioactive glasses in addition to TiO_2 (x = 0–3.0 wt %) were prepared by melt route. Glass–ceramics were prepared by controlled two-step heat treatment of the as-prepared Silica-based bioactive glasses at their nucleation and crystallization temperatures. Nucleation and crystallization temperatures were found from DTA experiment. X-ray diffraction (XRD) experiment is done to know the amorphous and crystalline nature of materials. The bioactivity of bioactive glasses and glass - ceramics was found by immersion of Bio Glasses and Glass- ceramics Samples in simulated body fluid (SBF) solution for different day and time by FTIR transmittance spectrometry with monitoring the pH changes and the concentration of Si, Na, Ca, P and Ti ions in SBF solution. Experimental results show that a increase in glass nucleation and crystallization temperature of 45S5 bioactive - glass by doping of TiO2 in it and the formation of crystalline phases of sodium calcium silicate and calcium titanium silicate in bioactive glass - ceramics. The bioactivity nearly remains same by doping 2% of TiO_2 by weight, but after that it decreases the bioactivity. Crystallization of bioactive glasses decreases the bioactivity.

Keywords: Bio ceramics, Bioactive 45S5 Glasses, Bioactive Glass - ceramics, Chemical Properties, Physical Properties, Bioactivity, SBF.

INTRODUCTION

For many years ago, it was thought that interactions between body and implants could cause only undesirable reactions, such as tissue pain, damage, and finally death. This was occurred if a toxic material is plant in contact with a living tissue, it will die. Due to this reason, the guiding principle used in development of biomaterial which should be chemically inert in nature as possible. [1]A bioactive material is one that elicits a specific biological response at the interface of the material which results in the formation of a bond between the tissues and the material". This definition was given by Hench (Hench et al.1994) who started this subject of research with his colleagues in the early 1970s [2]. They discovered that new compositions of glasses in the system SiO_2, CaO, Na_2O and P_2O_5 were able to form a bond with bone once they are implanted. In fact, when these glasses were plant in contact with biological fluids, a layer of hydroxyapatite (HA) analogue to the mineral phase of bones was deposited on their surface. Collagen molecules were integrated into this layer, and a biological bond could be formed. Later work by Wilson and Nolletti showed that a bond with soft tissue could be achieved too, if the speed of apatite construction was high enough [3]. The rate of bonding of bioactive glasses depends on many factors. One is bulk composition: the most rapid rates of bonding for bioactive glasses which have composition SiO_2, CaO, Na_2O and P_2O_5 are obtained with SiO_2 contents of 45-52% weight. In this compositional range, a bonding both to soft and hard connective tissue occurs very fast within 5-10 days. Bioactive glasses or glass ceramics containing 55-60% SiO_2 need a longer time to form a bond with bones, and do not bond to soft tissues. Glass compositions have more than 60% SiO_2 do not able to bond either to bone or to soft tissues, and obtain formation of a non adherent fibrous interfacial capsule [4].

Among the various modifying metallic ions, titanium ion is used in direct contact with living tissues [5]. The mechanical and chemical durability of phosphate based bio glasses is improved by the addition of TiO_2 in a certain amount.[6]

TiO_2, normally known to have good hemocompatiblity and non-toxicity to experimental in living tissue, which are very useful in clinical fields. Many literatures are available on phosphate-based bio glasses and glass - ceramics with different contents of TiO2 in order to investigate the physical, chemical, mechanical and biological properties of materials [7-11]. Application of glass–ceramics in the field medical is limited due to their inherent mechanical properties such as brittleness, low tensile strength, micro hardness, flexural strength and difficulty in coating onto other materials [12]. As our previous study [13], we have further proceed to next level in this research paper.

EXPERIMENTAL PROCEDURE:

(A) Synthesis of Materials:

(I) Preparation Of Tio_2 Doped 45S5 Bioactive Glasses:

The raw materials which are used to make TiO_2 Doped bio active glass samples are: SiO_2, Na_2O, CaO, P_2O_5, and TiO_2. These are obtained from following resources which are given in table (1)

Table1: Raw material resources for TiO_2 Doped bio glasses

S.N.	Constituents	Source materials
1	SiO_2	Quartz (AR)
2	Na_2O	Na_2CO_3 (AR)
3	CaO	$CaCO^3$ (AR)
4	P_2O_5	$NH_4H_2PO_4$ (AR)
5	TiO_2	TiO_2 (AR)

Acid washed silica was used as the source of SiO_2 while Na_2O and CaO were introduced in the form of anhydrous sodium carbonate [Na_2CO_3] and anhydrous calcium carbonate [$CaCO_3$] respectively, P_2O_5 was added in the form of ammonium dehydrogenate orthophosphate [$NH_4H_2PO_4$] and TiO_2 was added as such for preparation of bioactive glasses. All the batch materials were of analytical grade chemicals and used without further purification. The compositions of TiO_2 doped bioactive glasses are given in Table 2.

Table 2: The nominal composition of bioactive glasses and glass–ceramics in (wt %)

Sample Code for Bio glasses	SiO_2	CaO	Na_2O	P_2O_5	TiO_2
BG 0.0	45	24.5	24.5	6	-
BG 0.5	45	24.5	24.0	6	0.5
BG 1.0	45	24.5	23.5	6	1.0
BG 1.5	45	24.5	23.0	6	1.5
BG 2.0	45	24.5	22.5	6	2.0
BG 3.0	45	24.5	21.5	6	3.0

Collection of raw materials and then mixing it in mortar and pestle. After mixing batch formed and batch Loaded in alumina crucible and placed it in furnace at room temp. Then increase temp. Up to 1400 0C and hold for 3 hours. Melted glass cast in rectangular mould on preheated aluminum plate. After casting of melted glass annealing at 500^0C to room temperature. Then Cutting, grinding and polishing is performed on casted samples and then it ready for characterization.

(II) Preparation of TiO_2 doped 45S5 bioactive glass and glass -ceramics (GC):

To make the bioactive glass ceramics, the bioactive glass samples were heated in muffle furnace in two steps at the deduced temperatures and times as shown in table. These temperatures were obtained from differential thermal analysis (DTA) of bioactive glass samples. Every sample was first heated slowly for the nucleation and holds it for 6 hours. Then it was further heated to reach another temperature for the crystal growth and hold it for 3 hours, the sample was left for cooling from inside temperature to room temperature at a rate of 20 °c/h. The heat treatment schedule for the TiO_2 doped 45S5 bioactive glasses were given with the help of DTA plots of BG0.5, BG1.5, BG3.0 samples and a research paper [15].

Table 3: The heat treatment schedule for making the glass- Ceramics

	Nucleation		Growth	
Bio glass sample	Temp.(^{0}c)	Time in (hrs)	Temp.(°c)	Time in (hrs)
BG 0.0	540	6	730	3
BG 0.5	560	6	755	3
BG 1.0	572	6	775	3
BG 1.5	584	6	812	3
BG 2.0	591	6	816	3
BG 3.0	602	6	820	3

After the heat treatment, Bio glass samples were transformed in to glass- ceramics and denoted by **GC 0.0, GC 0.5, GC 1.0, GC 1.5, GC 2.0 and GC 3.0**.

(III) Preparation of simulated body fluid (SBF):

For the preparation of fresh 1000 ml SBF Solution , 950 ml of distilled water have taken and put it into 1000 ml polyethylene beaker. Heat the beaker at 37±10C temperature and dissolve 6.057 and adjust the pH value of the solution by adding 1M HCl at 37^0C. Add the reagents listed in Table 4 one by one in the order given from 1 to 8 with vigorous stirring. Then add de-ionized water to complete the mark of the beaker to 1000 ml and keeping the pH at 7.4. The SBF should be used within 30 days after preparation and being kept at 5-100 ^{0}C in a refrigerator [16]

Table 4. Reagents and its amount in (grams)

Order	**Reagents**	**Amount (grams)**
1	NaCl	7.996
2	$NaHCO_3$	0.350
3	KCl	0.224
4	$K_2HPO_4.3H_2O$	0.228
5	$MgCl_2.6H_2O$	0.305
6	IM HCl	40ml
7	$CaCl_2$	0.278
8	Na_2SO_4	0.071
9	$(CH_2OH)_3CNH_2$	6.057

(B) MEASUREMENT AND CHARACTERIZATION:

(I) Differential Thermal Analysis (DTA):

Differential thermal analysis (DTA) is mostly used for thermal analysis method. In DTA, the temperature of a sample is compared with that of an inert reference material during a programmed change of temperature. The temperature should be the same until thermal event occurs, such as melting, decomposition or change in the crystal structure. In an endothermic event takes place within the sample, the temperature of the sample will decrease behind that of the reference and a minimum will be observed on the plot. On the contrary, if an exothermic event takes place, then the temperature of the sample wills increases that of the reference and a maximum will be observed on the plot. The area under the endothermic is related to the enthalpy of the thermal event, ΔH. For many problems, it is advantageous to use both DTA and TG, because the DTA events can then be classified into those which do or do not involve mass change. DTA/TGA analysis of ash has been done by up to 1000 0C.

(II) X-ray Diffraction (XRD):

X-ray diffraction (XRD) is a multipurpose, nondestructive technique which is used to know crystal Structure of materials.

(III) Chemical and in vitro Bioactivity test:

Kokubo discovered the concept of in vitro bioactivity test (In 1991), which is carried out in simulated body fluid (SBF) instead of living body fluid, known as in vivo bioactivity test. The ion concentration of simulated body fluid is nearly same as that of human blood plasma.

(a) The ph Measurement: The pH value of the SBF solution before and after soaking of bioactive glass and glass – ceramics samples recorded by pH meter for 1day, 3 days, 7days and 15 days.

(b)Fourier Transforms Infrared Transmittance Spectroscopy:

Fourier Transform Infrared Spectroscopy (FTIR) is dominant instrument for identifying types of chemical bonds which is formed in a molecule by producing an infrared absorption spectrum that is like a molecular "fingerprint". It can be used to find out quantity of some compound of unknown mixture.

Table 5: Correlation between Wave number at which Transmittance Bands

Wavenumber(cm-1)	**Functional Groups**
400-500	Si-O-Si (bend)
500 - 560	P-O (Bend) (Crystalline)
560 - 600	P-O (Bend) (Amorphous)
720 - 840	Si-O-Si (Tetrahedral)
860 - 940	Si-O (Stretch)
1000 - 1100	Si-O-Si (Stretch)
1100 - 1200	P-O (Stretch)
1400 - 1530	C-O (Stretch)

Correlation between Spectral Frequencies and Functional Groups in a Bioactive Glass and their Ceramic Derivative and the Steps of Surface Changes by immersing in Simulated Body Fluid (SBF) is given in table 6. [36]

Table 6: Correlation between Wave number at which Transmittance Bands

Wavenumber(cm-1)	**Vibrational Mode**	**Surface reaction stages**
860 - 940	Si-O (Stretch)	Stage 1 and 2
720 - 840	Si-O-Si (Tetrahedral)	Stage 3
560 - 600	P-O(Bend) (Amorphous)	Stage 4
500 - 560	P-O(Bend) (Crystalline)	Stage 5

(C) RESULT AND DISCUSSIONS:

(I) Differential Thermal Analysis (DTA) Plot:

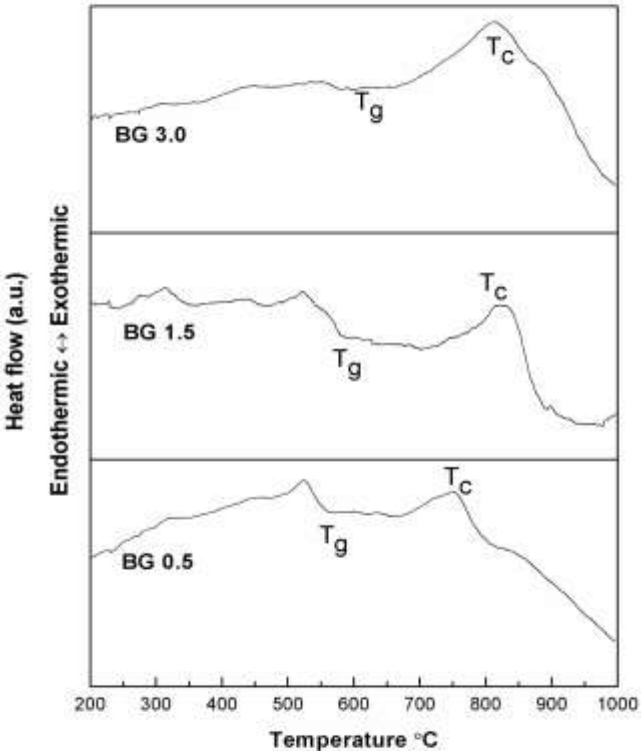

Figure 1: Differential thermal analysis (DTA) plot of TiO_2 doped 45S5 bioactive glasses samples

Tg - Nucleation Temperature, **Tc** - Crystallization Temperature

Discussion: The differential thermal analysis (DTA) curves of bioactive glasses show the glass transition temperature (endothermic peak) in the range of 540-597 ^{0}C and crystallization temperature (exothermic peak) in the range of 730-820^{0}C. The substitution of Na_2O with TiO_2 increases the glass transition temp. (Tg) and crystallization temperature (Tc) with increases the TiO_2 amount from 0.5 to 3.0%.

(ii) XRD of Bioactive glass (BG) and Glass – Ceramics (GC):

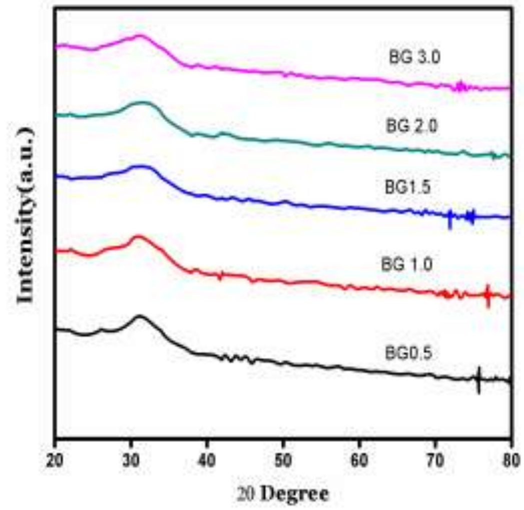

Figure 2: XRD of bioactive glass samples

Discussion: All prepared bioactive glass samples show amorphous in nature by XRD experiment of TiO_2 doped 45S5 bioactive glass samples because there was no peak found in XRD pattern, this shows our melting was homogeneous.

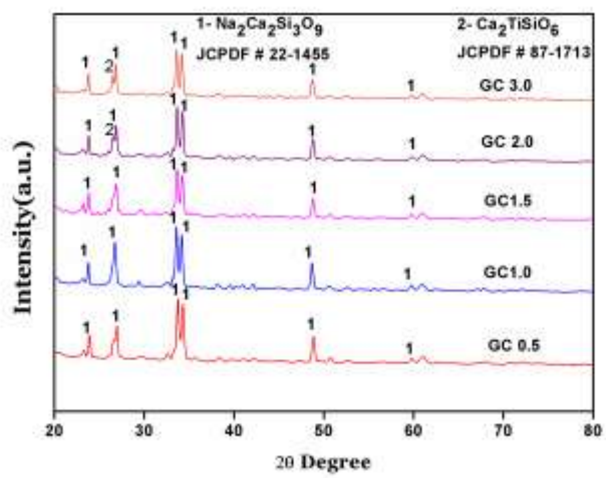

Figure 3: XRD of bioactive glass - ceramics samples

Discussion: The XRD patterns of all TiO_2 doped bioactive glass – ceramics samples show the presence of crystalline phases. The XRD result of glass – ceramic samples shows the sodium calcium silicate ($Na_2Ca_2Si_3O_9$) JCPDF # 22 - 1455 as the main Crystalline phase, by increase TiO2 amount a new phase calcium titanium silicate (Ca_2TiSiO_6) JCPDF # 87 - 1713 is found in samples GC 2.0 and GC 3.0

(III) Chemical and in vitro bioactivity test:
(a) pH Measurement:

pH measurement of simulated body fluid after soaking bioactive glass samples for different days:

Table7: pH value of SBF after soaking bioactive glass samples

Sample	0 day	1 day	3 day	7 day	15 day
BG 0.0	7.40	9.47	9.80	9.69	8.97
BG 0.5	7.40	8.83	9.71	9.60	9.04
BG 1.0	7.40	8.60	9.68	9.58	9.01
BG 1.5	7.40	8.15	9.57	9.40	8.68
BG 2.0	7.40	8.40	9.47	9.35	8.98
BG 3.0	7.40	8.30	9.39	9.10	8.84

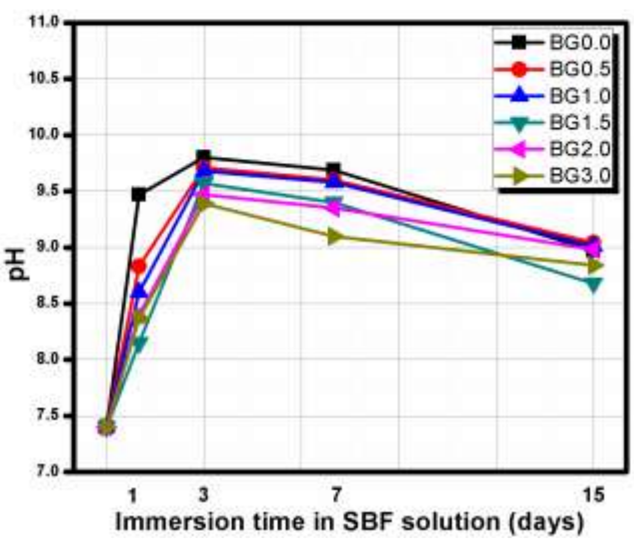

Figure 4: Variation of pH value of SBF after soaking bioactive glass samples

pH measurement of simulated body fluid after soaking bioactive glass Ceramics samples for different days:

Table 8: pH value of SBF after soaking bioactive glass ceramics samples

Sample	0 day	1 day	3 day	7 day	15 day
GC 0.0	7.40	8.42	9.76	9.54	8.32
GC 0.5	7.40	8.60	9.62	9.45	9.20
GC 1.0	7.40	8.86	9.51	9.31	9.18
GC 1.5	7.40	8.62	9.49	9.25	8.04
GC 2.0	7.40	8.52	9.31	9.98	8.60
GC 3.0	7.40	8.84	9.21	9.87	8.47

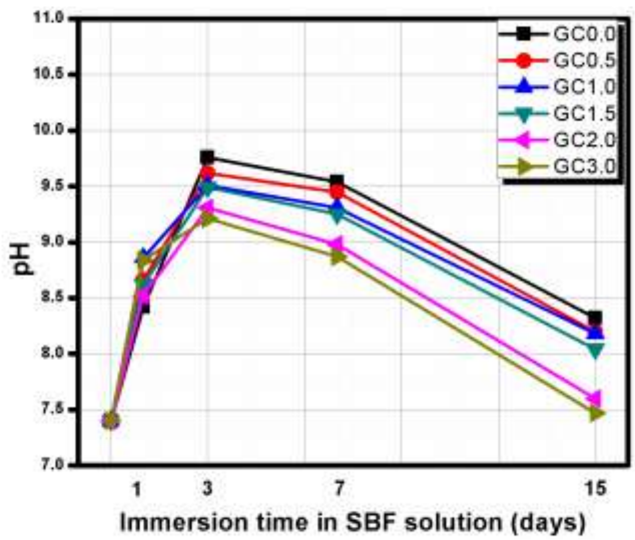

Figure 5: Variation of pH value of SBF after soaking glass ceramics samples

Discussion: The variation in pH value of simulated body fluid (SBF) solution due to soaking of TiO_2 bioactive glasses and glass-ceramics (figures) for different time is important because there should not more changes in pH value of human blood plasma by implantation of bioactive material. During starting period of soaking, faster release of Na and Ca ions increases the pH value of the simulated body fluid, but after that it mostly constant since the rate of release of Na ions decreased. Partial substitution of Na_2O by TiO_2 in base bioactive glasses causes a small decrement in pH of simulated body fluid. The Ph value of SBF after soaking the bioactive glass and glass ceramics are increases up to 3 days after that it decreases for 7 days and 15 days

(b) FTIR test of bioactive glass and glass Ceramics:

The Fourier transform infrared (FTIR) transmittance spectra of bioactive glass (BG) and glass – ceramics (GC) before and after soaking in simulated body fluid (SBF) for period of 1,7 and 15 days.

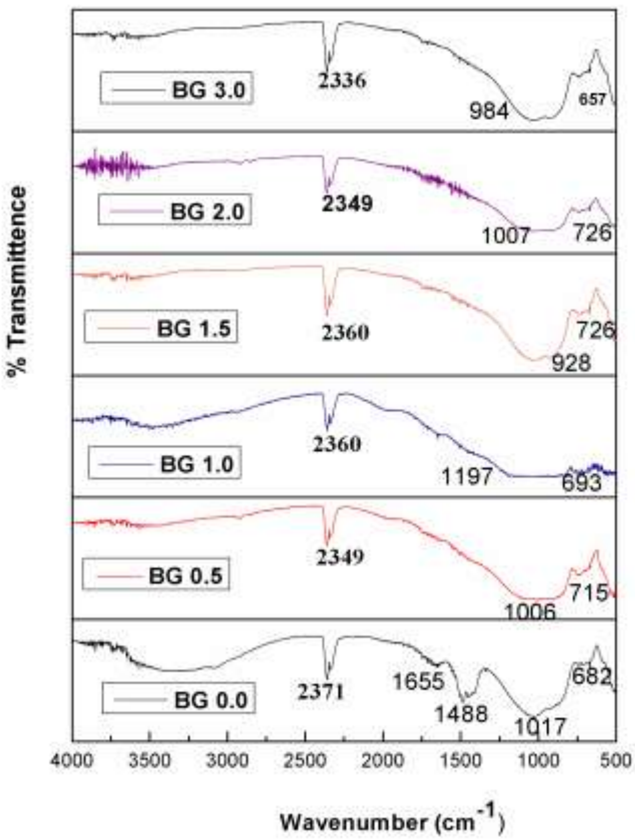

Figure 6: The FTIR transmittance spectra of bioactive glass samples before soaking in SBF solution.

Discussion:

The spectral bands in bio glass sample (BG 0.0) was found Si-H (2371 Cm^{-1}) , C=O (stretch) at wavenumber1655 Cm^{-1} , C-O(stretch) at wavenumber1488 Cm^{-1} , Si-O-Si (stretch) at wave number 1017Cm^{-1}.

The spectral bands of bio glass sample (BG 0.5) was found Si-H (2349 Cm^{-1}), Si-O-Si (stretch) at wavenumber1006 Cm^{-1}, Si-H , Si-O-Si (tetrahedral) at wave number 715 Cm-^{1}.

The spectral bands of bio glass sample (BG 1.0) was found Si-H (2360 Cm^{-1}) , P-O (stretch) at wavenumber1197 Cm^{-1} , Si-O-Si (tetrahedral) at wave number 693 Cm^{-1}.The spectral bands of bio glass sample (BG 1.5) was found Si-H (2360 Cm^{-1}) , Si-O (stretch) at wave number 928 Cm^{-1}, Si-O-Si (tetrahedral) at wave number 726 Cm^{-1}.The spectral bands of bio glass sample (BG 2.0) was found Si-H (2349 Cm^{-1}) , Si-O-Si (stretch) at wavenumber1007 Cm^{-1}, Si-O-Si (tetrahedral) at wave number 726 Cm^{-1}.The spectral bands of bio glass sample (BG 3.0) was found Si-H (2336 Cm^{-1}) , Si-O-Si (stretch) at wave number 984 Cm^{-1}, Si-O-Si (tetrahedral) at wave number 657 Cm^{-1} shown in figure 6.

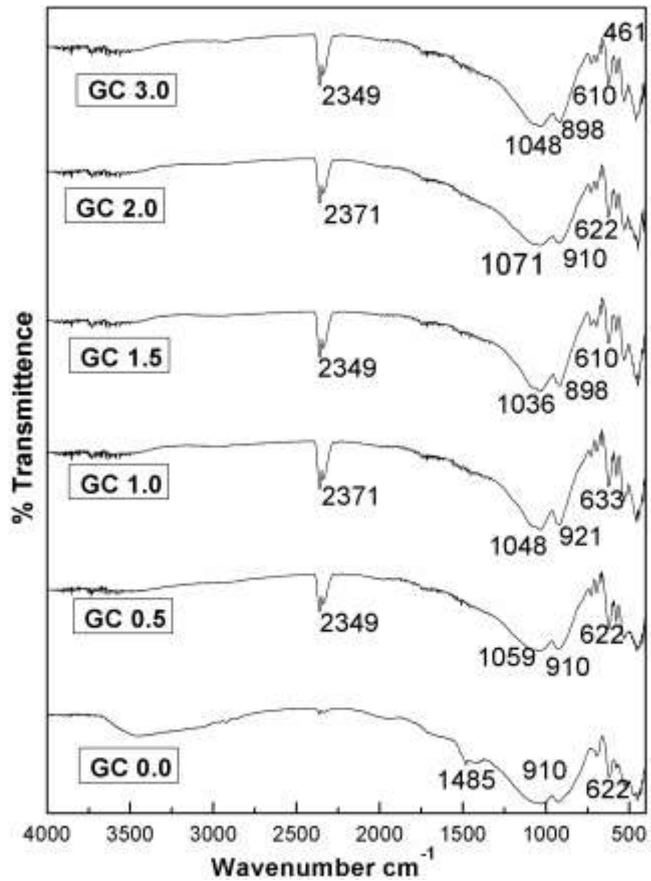

Figure7: FTIR transmittance spectra of bio glass-ceramic samples before soaking in SBF solution

Discussion: The spectral bands found in all glass ceramics samples were Si-H (2349, 2371), Si-O-Si (stretch) at wave numbers (1048, 1059, 1071 and 1036), Si-O (stretch) at wave number (898, 910, 921) and Si-O-Si (bend) were found in all the glass ceramics samples at wave number (461) shown in figure 7.

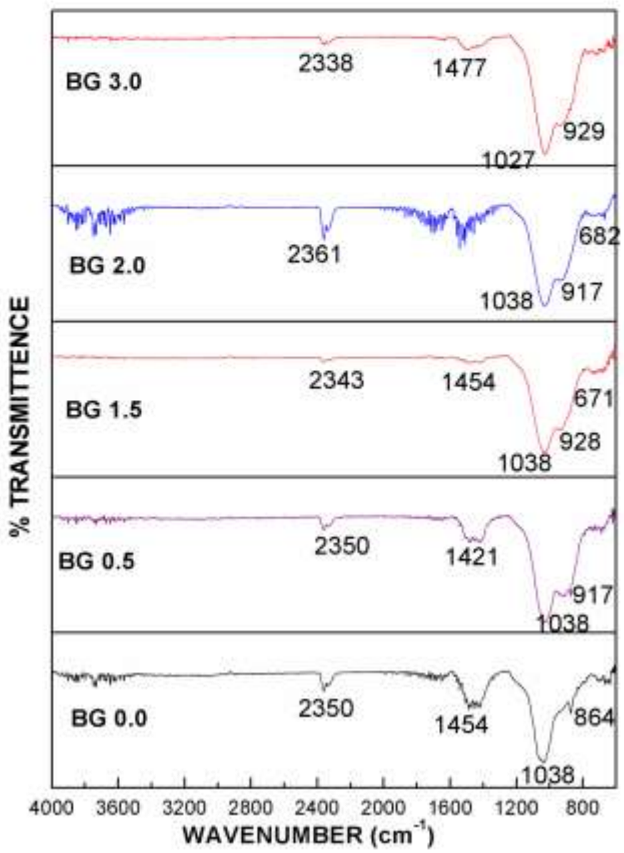

Figure 8: FTIR transmittance spectra of bio glass samples after soaking for a period of 1 day in SBF solution.

Discussion: FTIR transmittance spectra of bio glass samples after soaking for a period of 1 day in SBF solution are shown in figure5.7 .There were found a sharp peak of Si-O-Si (stretch) at wave numbers (1027,1038) in all samples. C-O (stretch) was found at wave numbers (1477, 1421, and 1454) and its peak is small broad and it decreases from BG 0.0 to BG 3.0. Si-O (stretch) was found at wave numbers (917, 928 and 929) in all samples except of BG0.0 shown in figure 8.

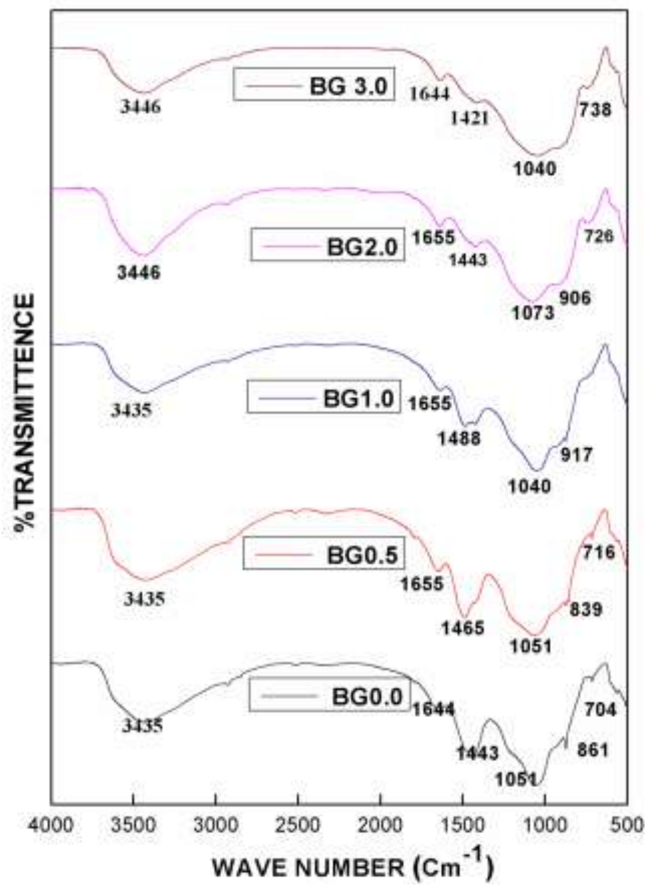

Figure 9: FTIR transmittance spectra of bio glass samples after soaking for a period of 7 days in SBF solution.

Discussion: FTIR transmittance spectra of bio glass samples after soaking for a period of 7 days in SBF solution shown in the figure 5.8. O-H (stretch) bands are found in all samples at wave numbers (3435, 3446). C=O (stretch) bands were found at wave numbers (1644, 1655). C-O (stretch) bands were found at wave numbers (1421, 1443, 1488, 1465). The intensity of C-O (stretch) bands was decreases from BG0.0 to BG3.0. Si-O-Si (stretch) bands were found at wave numbers (1040, 1051, and 1073) and its intensity was decreases from BG0.0 to BG3.0. Si-O (stretch) bands were found at wave numbers (906, 917, 839, and 861). There were found a sharp peak of Si-O-Si (Tetrahedral) at wave numbers (716,726,738,704) in all samples shown in figure 9.

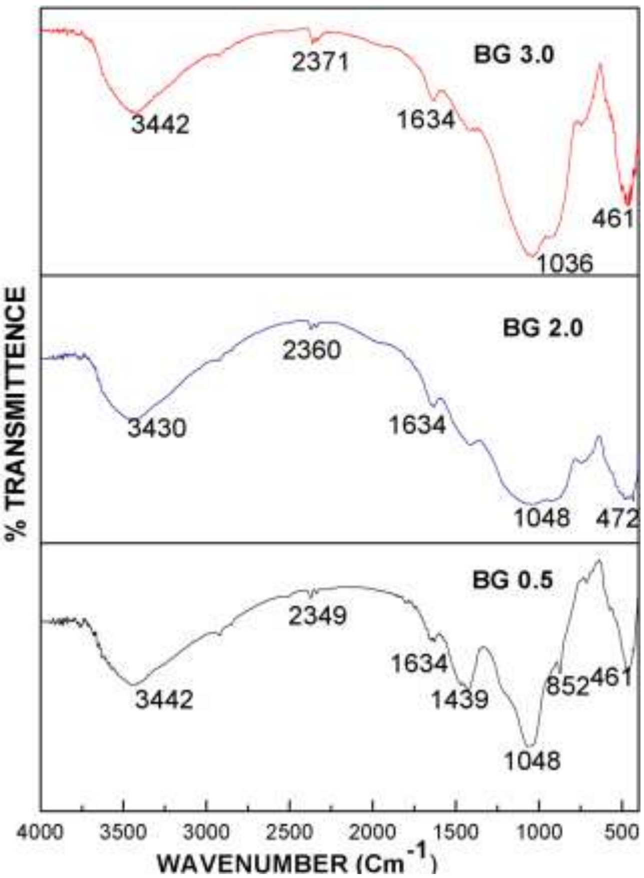

Figure 10: FTIR transmittance spectra of bio glass samples after soaking for a period of 15 days in SBF solution.

Discussion: FTIR transmittance spectra of bio glass samples after soaking for a period of 15 days in SBF solution shown in the figure 5.9. O-H (stretch) bands are found in all samples at wave numbers (3430, 3442). C=O (stretch) bands were found at wave number (1634) in all bio glass samples. C-O (stretch) bands were found at wave number (1439) only BG 0.5 samples. Si-O-Si (stretch) bands were found at wave numbers (1048, 1036) in bio glass samples and its intensity was maximum for BG0.0 and BG3.0. Si-O-Si (bend) bands were found at wave numbers (461,472) in all bio glass samples. Si-O (stretch) bands were found at wave number (852) in BG 0.0 samples shown in figure 10.

CONCLUSIONS:

- All the bio glass samples could be successfully prepared for characterization purpose by melt route.
- On increasing the substitution of TiO_2 in place of Na_2O, the nucleation and crystallization temperature increases with increasing amount of TiO2.
- All prepared bio glass samples were found amorphous by XRD experiments.
- The Ph value of SBF after soaking the bioactive glass and glass ceramics are increases up to 3days after that it decreases for 7 days and 15 days.
- The FTIR transmittance spectra showed different characteristic bands due to silicate network with measurable modifiers and non bridging oxygen ions which indicate the hydroxy calcium apatite (HCA) layer on prepared bioactive glass ceramic samples after immersion of in SBF from 1 to 15 days.

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