Effect of Doping Agent on the Physico-chemical properties of 45S5 Bioactive Glass

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Abstract

Any material incorporated into a human organism has to abide by certain properties that will assure that there are no negative interactions with living tissue. Biomaterials by definition are inorganic compounds that are designed to replace a part or a function of the human body in a safe, reliable, economic, and physiologically and aesthetically acceptable manner. In present investigation, the primary aim of the project was to develop better bioglass-ceramics, which are bio-chemically compatible and non-toxic and to study the effect of doping agent on the physic-chemical properties of **45S5** bioactive glass. The results of preparation, characterization and in vitro bioactivity evaluation of phosphosilicate glasses based on Bioglass_ 45S5 (SiO_2 45; Na_2O 24.5; CaO 24.5; P_2O_5 6 wt %) doped during melting with (0.5–8wt %) cerium dioxide (CeO_2), has been reported. The choice of cerium was related to its low toxicity associated with bacteriostatic properties; cerium-doped bioactive glasses could be useful when implantation concerns local infected areas.

***Key words**:* Bioactive glass, Cerium, Doping agent

1. Introduction

In 1969, the concept of bioactive material was discovered [1] and since then, the field of bioactive ceramics has expanded to include a great variety of compositions [1–3] and various kinds of glasses and glass-ceramics have been found to bond to living bone [4].Bonding to bone was first demonstrated by Hench [2] for a certain compositional range of glasses which contained SiO_2, Na_2O, CaO, P_2Oin specific proportion. The first and well-studied composition was B_5 bioglass_ 45S5, which contains45% SiO_2, 24.5% Na_2O, 24.5% CaO and 6% P_2O_5, in weight percent. Other bioactive glasses are derived from Bioglass_ (e.g. 45S5F, 45B15S5, 52S4.6and 55S4.3Bioglass_). The surface-active bone bonding glass formulations can also be produced by glass-ceramics by nucleation and growth of crystals in the glass. They can be subdivided in two groups: the apatite–wollastonite group developed by Kokubo et al. [5] (Ceravital_, Cerabone_A-W,Ilmaplant_L1) and mica-based materials originally synthesized by Beall et al. [6] and later also by Vogel et al.(Bioverit_) [7].Kokubo and co-workers [4] reported that alsoP_2O_5-free CaO–SiO_2 glasses bond to living bone and a biologically active apatite layer can be formed by P_2O_5-free Na_2O–SiO_2 glasses; it was also reported that there is a little difference betweenP_2O_5-containing Bioglass_ 45S5-type and a corresponding P_2O_5-free Na_2O–CaO–SiO_2 glasses in the rate of apatite formation. The common characteristic of bioactive ceramics is a time-dependent, kinetic modification on the surface that occurs upon implantation [2].They form, when exposed to physiological solutions, an amorphous calcium-phosphate layer, on the surface; this layer crystallizes into a biologically active hydroxycarbonate apatite layer (HCA)[2,4] in a few days. This phase is chemically and structurally equivalent to the mineral phase in bone and is responsible for interfacial bonding. Bonding to bone [2] involves 12 reaction stages: the first five reaction stages do not depend on the presence of tissues and result in a HCA crystalline layer formed on the implant surface, the subsequent stages are necessary for the implant to bond to tissues. It has been demonstrated that the same kind of apatite layer can be formed on the surfaces of bioactive glasses and glass-ceramics also in an cellular simulated body fluid (SBF) with ion concentrations nearly equal to those of human blood plasma Recently Agdopedglasses were reported: a new composition of sol–gel derived bioactive glass denoted AgBG (which composition determined by EDS was $SiO_2$76; CaO 19; P_2O_5 2; Ag_2O 3 wt%) featuring unique antibacterial properties.

Similar experiments were attempted in our laboratory but they were unsuccessfully because silver ion seemed to be reduced to elemental silver and formed an alloy with Alumina crucible so giving rise to badly reproducible\glass compositions and a very low %age were obtained (<0.7 wt%) . The manufacture of cerium containing glasses does not present the same problems and cerium displays bacteriostatic properties such as silver (it has been reported that the activity of cerium (III) sulfadiazine against various microorganisms is comparable to that of silver sulfadiazine) and cerium-doped bioactive glasses could be useful when implantation concerns with periodontal pockets, infected frontal sinuses and hypersensitive teeth as a complication of periodontal treatment or tooth wear that has resulted in the exposure of dentin and dental tubules[8]. In addition cerium present a low toxicity (e.g. ingestion of CeO_2 for a rat is acceptable until 1000 mg/kg of body weight [9]).Here we report the results on preparation, characterization and in vitro bioactivity of phosphorsilicate glasses based on Bio-glass 45S5 doped with cerium dioxide (CeO_2), during melting up to the maximum value of 10 wt%, that permitted to obtain a homogeneous glass and to get a better insight into CeO_2 effect on the chemical behavior glasses.

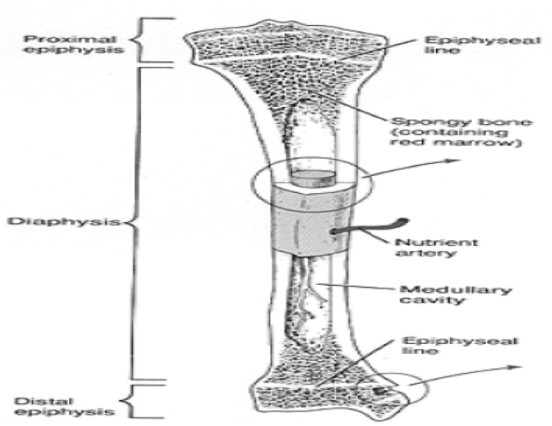

Figure:- 1 The structure of bone

2. Classification of biomaterials according to the interface formed with tissue

Any material incorporated into a human organism has to abide by certain properties that will assure that there are no negative interactions with living tissue. Biomaterials by definition are inorganic compounds that are designed to replace a part or a function of the human body in a safe, reliable, economic, and physiologically and aesthetically acceptable manner (Hench and Ethridge, 1982). Since biomaterials are inorganic Structures they do not include renewable materials obtained from natural sources such as wood, plant fibers, hides, sinew, bone, ivory and others. One of the important properties of biomaterials is their so-called ***biocompatibility.***

Incompatible materials are materials that release to the body substances in toxic concentrations and/or trigger the formation of antigens that may cause immune reactions ranging from simple allergies to inflammation to septic rejection with the associated severe health consequences.

Biocompatible materials, in contrast, are those that also release substances but in non-toxic concentrations that may lead to only benign tissue reactions such as formation of a fibrous connective tissue capsule or weak immune reactions that cause formation of giant cells or phagocytes. These materials are often called ***biotolerant*** and include austenitic stainless steels or bone cement consisting of polymethylmethacrylate (PMMA).

Bioinert materials do not release any toxic constituents but also do not show positive interaction with living tissue. As a response of the body to these materials usually a non-adherent capsule of connective tissue is formed around the bioinert material that in the case of bone remodeling manifests itself by a shape-mediated contact ontogenesis. Through the bone-materials interface only compressive forces will be transmitted ("bony on-growth"). Typical bioinert materials are titanium and its alloys, ceramics such as Alumina, Zirconium and Titanium, and some polymers, as well as carbon.

Bioactive materials show a positive interaction with living tissue that includes also differentiation of immature cells towards bone cells. In contrast to bioinert materials there is chemical bonding to the bone

along the interface, thought to be triggered by the adsorption of bone growth-mediating proteins at the biomaterials surface. Hence there will be a biochemically-mediated strong bonding ontogenesis. In addition to compressive forces, to some degree tensile and shear forces can also be transmitted through the interface ("bony in growth").

Resorbable material represents an alternative solution to the problem of long-term implant failure. These materials are supposed to exploit and increase the body capacity of self repairing. This happens as these materials degrade gradually over a period of time, and are replaced by the natural host tissue. An important issue is the biocompatibility of the products of resorption. Moreover, resorption should occur at a rate similar to cellular metabolism. These requirements are very difficult to be fulfilled, and for this reason not very many resorbable biomaterials are clinically applied yet.

3.Material and Method

Batch making: Composition Selection**,** Formula- (45S5-X)+XCeO2 ,X=0.5,2,3,4,6,8

45S5 Composition (45SiO_2, 24.5Na_2O, 24.5CaO, 6P_2O_5 wt %)

Preparation of Glass powder: Four different types of doped glasses were prepared, and as reference, a glass with the composition corresponding to Bioglass_ **45S5** (hereafter as BG) was also prepared, as reported in Table 3.1. About 100 g of batch were prepared by mixing reagent grade Na_2CO_3, $CaCO_3$, Na_3 PO_4 $12H_2O$, SiO_2 and CeO_2, raw materials in a sealed polyethylene bottle for 1 h. Premixed batches were put into a 50 ml alumina crucible and melted in an electric oven for 1 h at 1400 ^oC; the samples with CeO_2 (0.5% and 3.2%) were melted at 1400 ^oC for 1 h and re-melted for 3 h (to ensure the homogeneity of the material). The sample with 8% of CeO2 were respectively melted at 1400 ^oC for 1 h (re-melted for 3 h) and 4h. The melts were poured, crushed and sieved through 25 mesh screen to produce fine particles.

Table 1 Experimental compositions of the examined glasses (wt %)

BG- Bioglass sample

	BG-1	BG-2	BG-3	BG-4	BG-5	BG-6	BG-7
CeO_2		0.5	2.00	3.00	4.00	6.00	8.00
SiO_2	45.0	44.7	44.1	43.6	43.2	42.3	41.4
Na_2O	24.5	24.3	24.0	23.7	23.5	23.0	22.5
CaO	24.5	24.3	24.0	23.7	23.5	23.0	22.5
P_2O_5	6.0	5.8	5.8	5.8	5.7	5.6	5.5

Composition of Sample BG-1 - 45 SiO_2, 24.5CaO, 24.5Na_2O, 6P_2O_5 ,BG-2 - 44.7 SiO_2, 24.3CaO, 24.3Na_2O, 5.8P_2O_5, 0.5CeO_2 ,BG-3 - 44.1 SiO_2,24CaO,24Na_2O,5.8P_2O_5 ,2CeO_2 ,BG-4 – 43.6 SiO_2, 23.7CaO, 23.7Na_2O, 5.8P_2O_5, 3CeO_2 ,BG-5 – 43.2 SiO_2, 23.5CaO, 23.5Na_2O, 5.7P_2O_5, 4CeO_2 ,BG-6 – 42.3 SiO_2, 23CaO, 23Na_2O, 5.6P_2O_5, 6CeO_2,BG-7 - 41.4 SiO_2, 22.5CaO, 22.5Na_2O, 5.5P_2O_5, 8CeO_2

4.Results And Discussion

Density measurement:

Table No.2 Density of different Bioactive glass samples

S.No.	Glass Composition (mole%)	CeO_2/CaO	CeO_2/ P_2O_5	Density (gm/cc)
1	$45SiO_2 24.5Na_2O 24.5CaO$ $6P_2O_5$	0	0	2.74
2	$44.7SiO_2 24.3Na_2O 23.7CaO$ $5.9P_2O_5$ $0.5CeO_2$	0.02	0.08	2.76
3	$44.1SiO_2 24.0Na_2O 24.0CaO$ $5.8P_2O_5$ $2CeO_2$	0.08	0.34	2.81
4	$43.6SiO_2 23.7Na_2O 23.7CaO$ $5.8P_2O_5$ $3CeO_2$	0.12	0.51	2.86
5	$43.2SiO_2 23.5Na_2O 23.5CaO 5.7P_2O_5$ $4CeO_2$	0.17	0.68	2.89
6	$42.3SiO_2 23CaO 23Na_2O$ $5.6P_2O_5$ $6CeO_2$	0.26	1.07	2.95
7	$41.4SiO_2 22.5CaO 22.5Na_2O$ $5.5P_2O_5$ $8CeO_2$	0.35	1.45	3.21

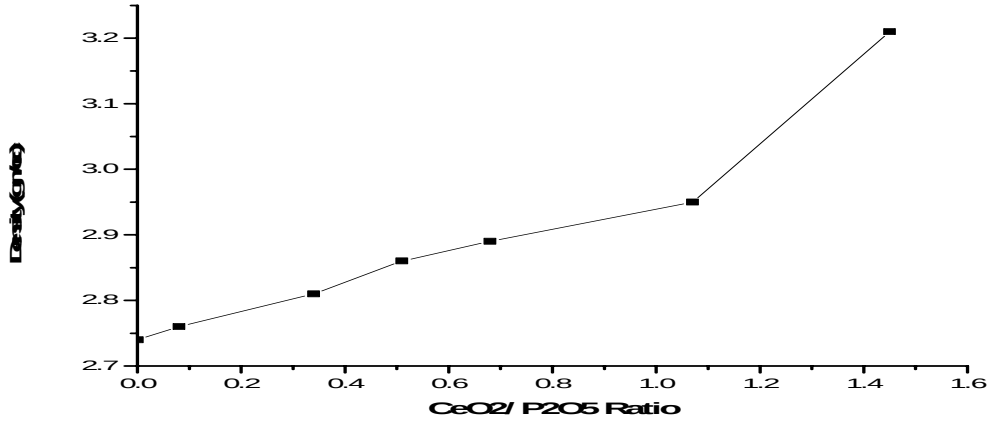

Fig.2 Variation of density of the glass with itsCeO_2/P_2O_5 Ratio

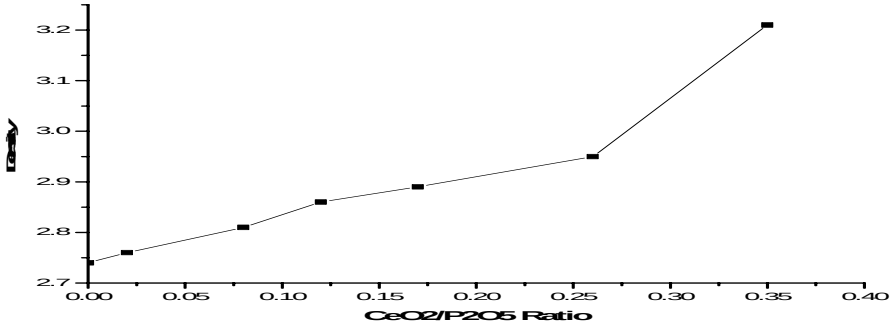

Fig.3 Variation of density of the glass with itsCeO_2/CaO Ratio

It is evident from fig.2and fig 3 and table 2 that the density of the glass has increased with an increase in CeO_2/CaO ratio and ratio CeO_2/P_2O_5 in the 45S5 bioactive glasses . It indicates that the replacement of a smaller Ca^{2+} ion by a heavier Ce^{3+} ion increases the density of the glass and the system is closely packed.

Compressive strength measurement

Table No3: Compressive Strength of different Bio active glass samples

S. No.	Glass Composition (mole%)	CeO_2/CaO	CeO_2/P_2O_5	Compressive Strength (M.Pa)
1	$45SiO_2 24.5Na_2O 24.5CaO$ $6P_2O_5$	0	0	155
2	$44.7SiO_2 24.3Na_2O 23.7CaO$ $5.9P_2O_5$ $0.5CeO_2$	0.02	0.08	160
3	$44.1SiO_2 24.0Na_2O 24.0CaO$ $5.8P_2O_5$ $2CeO_2$	0.08	0.34	176
4	$43.6SiO_2 23.7Na_2O 23.7CaO$ $5.8P_2O_5$ $3CeO_2$	0.12	0.51	180
5	$43.2SiO_2 23.5Na_2O 23.5CaO 5.7P_2O_5$ $4CeO_2$	0.17	0.68	183
6	$42.3SiO_2 23CaO 23Na_2O$ $5.6P_2O_5$ $6CeO_2$	0.26	1.07	194
7	$41.4SiO_2 22.5CaO 22.5Na_2O$ $5.5P_2O_5$ $8CeO_2$	0.35	1.45	210

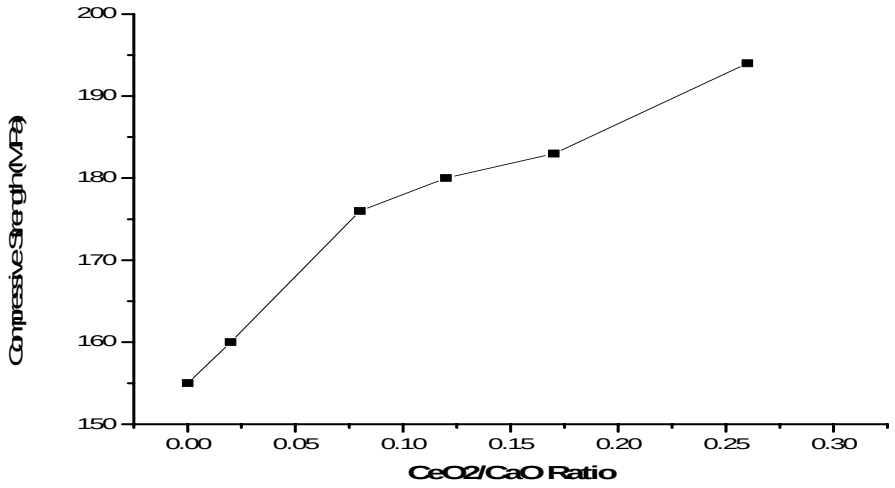

Fig.4. Variation of compressive strength with CeO_2/CaO Ratio

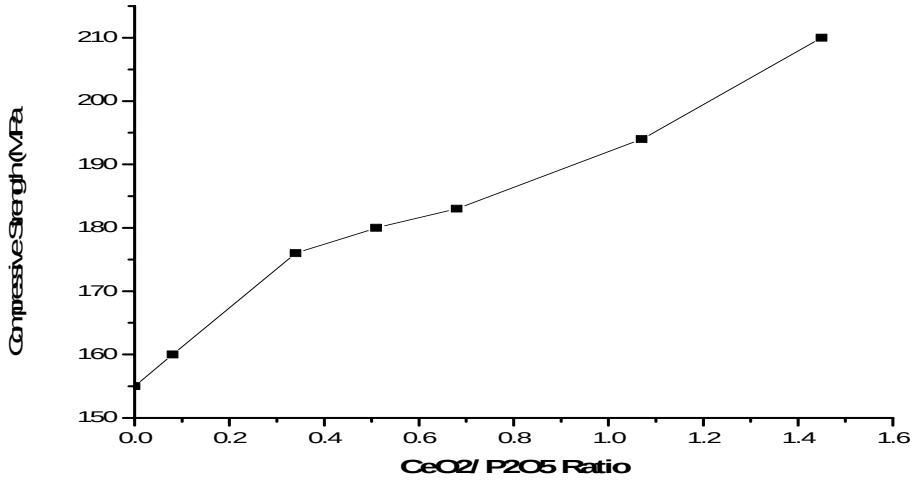

Fig.5 Variation of compressive strength with CeO_2/P_2O_5 Ratio

It is evident from fig.4.and fig 5 and table 3 that the compressive strength of the bio glass has increased with an increase in CeO_2/CaO ratio and ratio CeO_2 /P_2O_5 in the 45S5 bio active glasses.

X-Ray Diffraction X-ray diffraction (XRD) is a versatile, non-destructive technique that reveals detailed information about the chemical composition and crystallographic structure of natural and manufactured materials. The X-ray radiation most commonly used is that emitted by copper, whose characteristic wavelength for the K radiation is =1.5418Å.

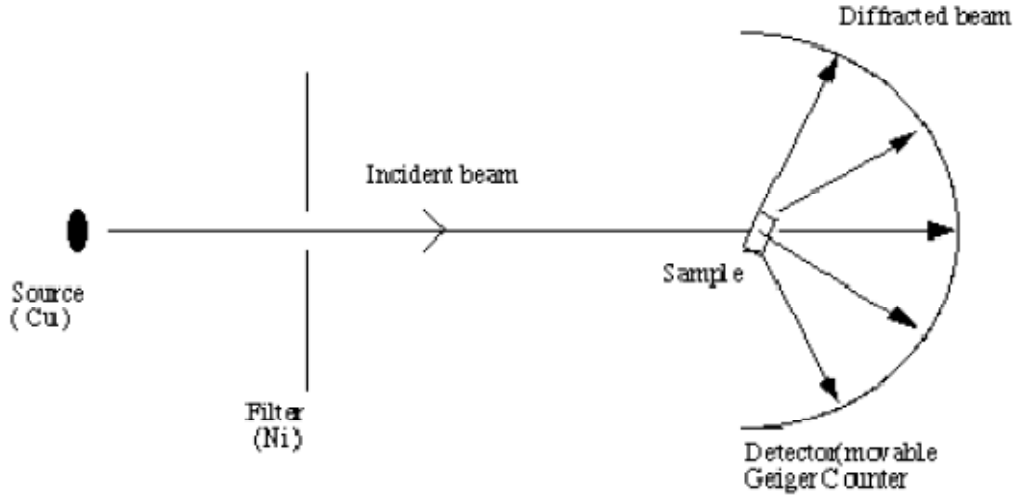

Fig.6 - Mechanism of X-ray diffraction.

The XRD pattern of the powdered glass samples shows that there was no XRD peak for the glass. It dictates that all the $SiO_2Na_2OCaOP_2O_5$ and CeO_2 Doped 45S5 glass samples have been melted properly and converted into glassy solids.

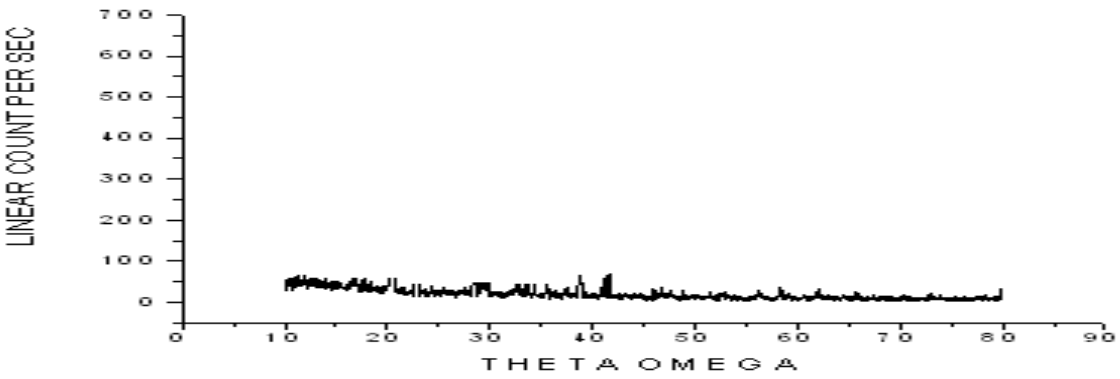

Fig. 7 XRD peak for 45S5 bio-glass sample

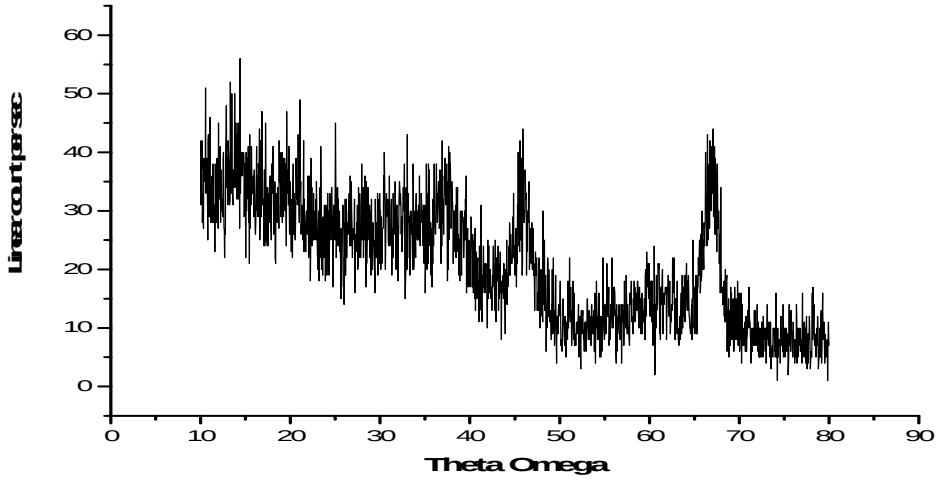

Fig 8 XRD peak for 45S5 (0.5gm) Cerium Doped bio-glass sampl

The specimens BG-1, BG-2,BG-3,BG-4,BG-5,BG-6,BG-7 were soaked in an cellular simulated body fluid (SBF,100ml) with ion concentration and ph nearly equal to those of human blood plasma. The SBF was prepared according to Kokubo et al. by dissolving reagent grade NaCl, $NaHCO_3$,KCl, $K_2HPO_4.3H_2O$, $MgCl_2.6H_2O$, $CaCl_2$ and Na_2SO_4 in an ion exchanged water contained in a polystyrene bottle. These reagents were added in the order they are listed. The solution was buffered at pH value 7.25 with 50 mM of trishydroxymethyl-aminomethane $(CH_2OH)_3CNH_2$ hereafter as TRIS) and 45 mM hydrogen chloride and its temperature was kept at 37 C.The soaking was carried out at 37 C, under continuous stirring and for various times (4 and 16 days)

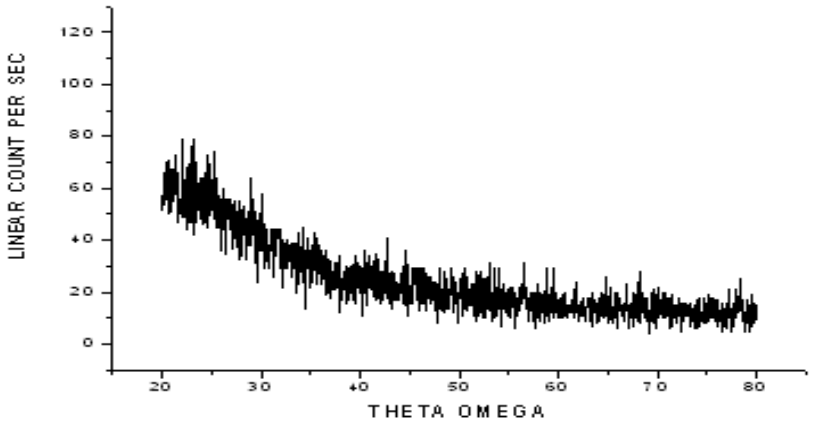

Fig 9 XRD peak for 45S5 (0.5gm) Cerium Doped bio-glass sample after SBF 4 Day

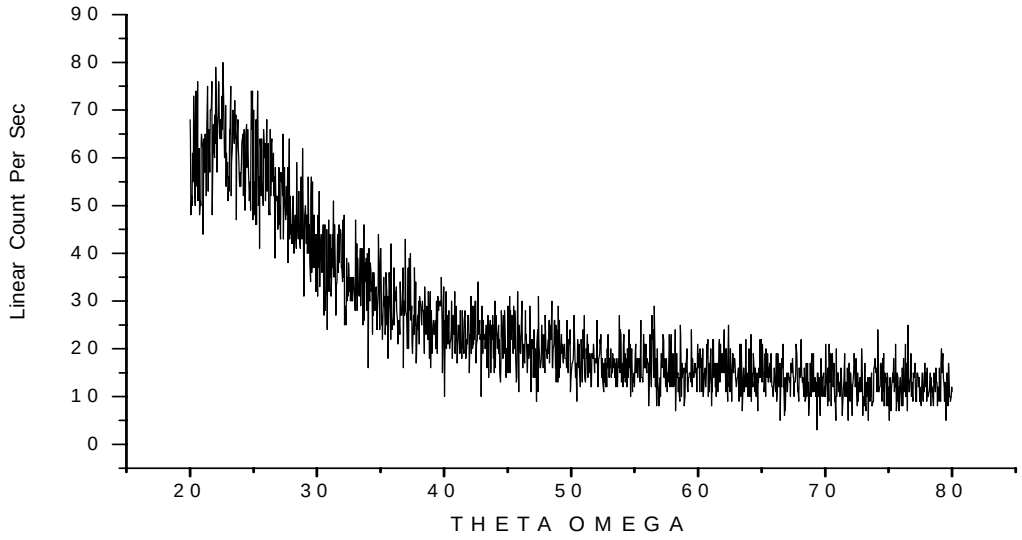

Fig 10- XRD peak for 45S5 (4 gm) Cerium Doped bio-glass sample after SBF 4 Day

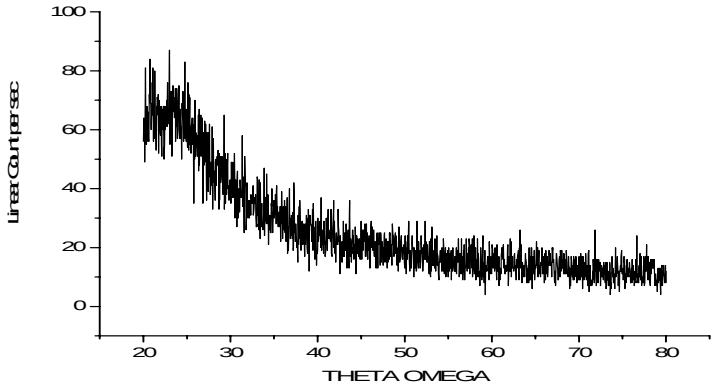

Fig 11- XRD peak for 45S5 (3 gm) Cerium Doped bio-glass sample after SBF 16 Day

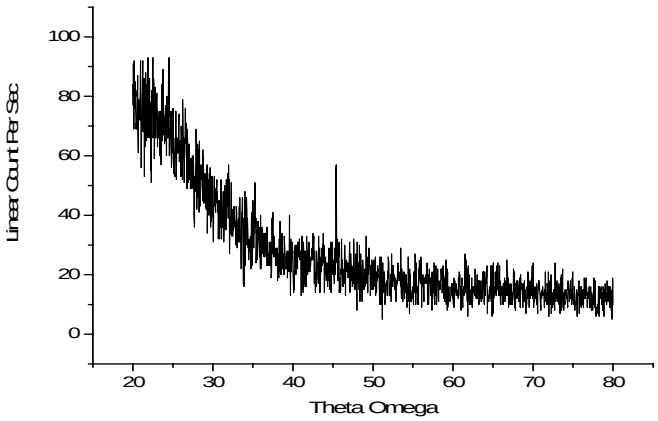

Fig 12- XRD peak for 45S5 (8 gm) Cerium Doped bio-glass sample after SBF 16 Day

Result

After SBF the XRD pattern of the powdered glass samples shows that there was no XRD peak for the glass. It dictates that all the $SiO_2Na_2OCaOP_2O_5$ and CeO_2 Doped 45S5 glass samples have been melted properly and converted into glassy solids.

5.Fourier Transform Infrared Spectroscopy (FTIR)

Fourier Transform Infrared Spectroscopy (FTIR) is powerful tool for identifying types of chemical bonds in a molecule by producing an infrared absorption spectrum that is like a molecular "fingerprint". It can be utilized to quantitative some compound of unknown mixture. It can be applied to his analysis of solids, liquid and gases. Infrared properties of glass and glass ceramics have been studied since the beginning of infrared spectroscopy some fifty year ago. The structure of solid may be completely described by three sets of data- chemical constitution, geometry of the atomic arrangement and the system of forces representing the interatomic bonds.

Molecular bonds vibrate at various frequencies depending on element and type of bonds. For any given bond, there are several specific frequencies at which it can be vibrate. According the quantum mechanics, these frequencies correspond to the ground state (lowest frequency) and several excited states (higher frequencies).One way to cause the frequency of a molecular vibration to increase is to excite the bond by having it absorb light energy. For any given transition between two states the light energy (determined by the wavelength) must exactly equal the difference in the energy between the two states [usually ground state (E_0) and the first excited

Difference in Energy State = Energy of Light Absorbed

$$\mathbf{E_1 - E_0 = chi/\lambda}$$

Where h=Planks constant
C=speed of light, and
λ= the wavelength of light

The energy corresponding to these transitions between molecular vibration states is generally 1-10 kilocalories/mole, which corresponds to the infrared portion of the electromagnetic spectrum. Most of the instruments are of the prism type and selecting a prism of suitable material can cover the desired working region. A NaCl prism is suitable between 2.5 and 15 micron and a KBr prism between 5 and 25 micron, the working range being limited on the short-wavelength side by the absorption in his prism. Other prism material such as CaF_2(1 to 9 micron), KRS-5(25 to40 micron)are also used. High resolving power is seldom needed in this work because most of the bands observed in glass ceramics are rather broad; high radiation efficiency is desirable, however, because the signal is often weak, particularly in reflection measurement and at long wavelength. The principle problem in the infrared spectroscopy of glass ceramics remains with the sample. The extinction coefficient (K_0=nK) attains a value of the order of unity in some

of the strong absorption bands in glass ceramics so that penetration depth (for electronics transmittance) become very small, typically about one micron at 10 micron wavelength.
Sample FTIR can be prepared in a number of ways. For liquid samples, the easiest is to place one drop of sample between two plates of sodium chloride (salt). Salt is transparent to infrared light. The drop forms a thin film between the plates. Solid sample can be milled with (KBr) to form a very fine powder. This powder is then co pressed into thin pallet, which can be analysed.KBr is also transparent in the IR.

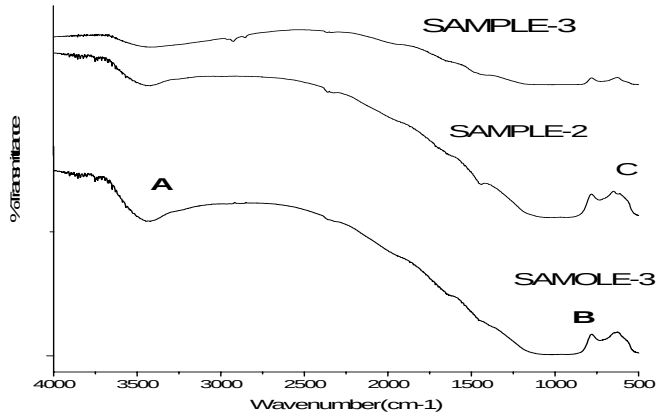

Fig 13 FTIR Transmission Bio-glass BG-7,BG-5,BG

The IR spectra, in the spectral range 4000–400cm_1, were performed on KBr pellets as support. The pellets were prepared with ca. 2 mg of sample and 98 mg of KBr.

SAMPLE-1:-8gm cerium-doped bio glass
SAMPLE-2:-4gm cerium-doped bio glass
SAMPLE-3:-Without doped bio glass

Table No.4-FTIR Transmission Bonds of Bio-glass BG-7,BG-5,BG

Vibrational Mode	Wave number(cm-1)
H-O-H(A)	3443.303
P-O (B)	935.664
P-O-P(C)	730.884

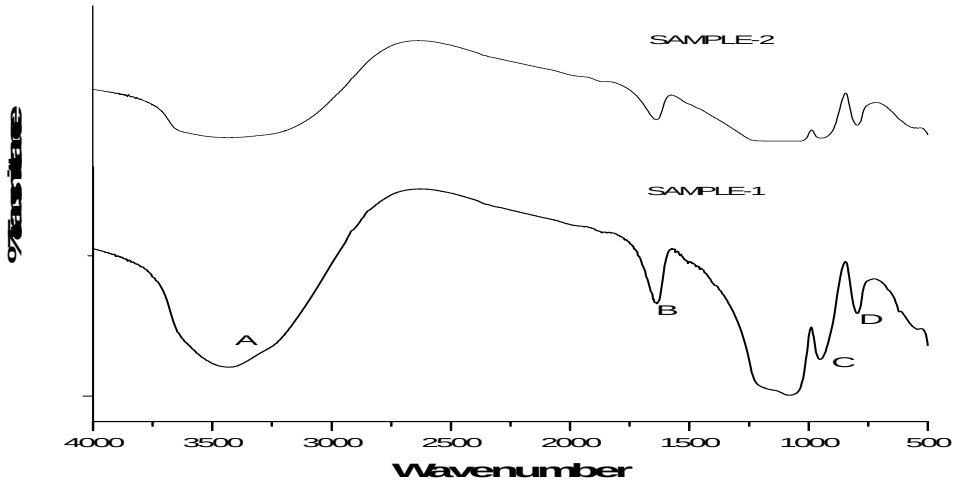

Fig 14- FTIR Transmission Bio-glass BG-5,BG-3

Spectroscopic (IR and–VIS) techniques were performed on the bio- glasses after soaking in SBF solution 4 day

SAMPLE-1:-4gm cerium-doped bio glass
SAMPLE-2:-2gm cerium-doped bio glass

Table No.5-FTIR Transmission Bonds of Bio-glass BG-3,BG-5

	Vibrational Mode	Wave number(cm-1)
A	**H-O-H**	**3433.613**
B	**Si-O**	**1081.915**
C	**P-O**	**952.407**
D	**Si-O(NBO)**	**796.208**

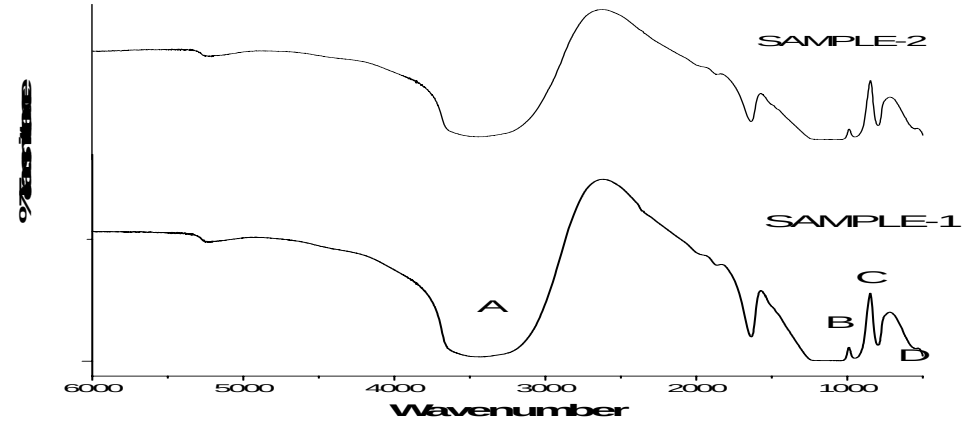

Fig 15- FTIR Transmission Bio-glass BG-7,BG-6

Spectroscopic (IR and–VIS) techniques were performed on the bio- glasses after soaking in SBF solution 16 day

SAMPLE-1:-8 gm cerium-doped bio glass SAMPLE-2:-6 gm cerium-doped bio glass

Table No.6-FTIR Transmission Bonds of Bio-glass BG-7,BG-6

	Vibrational Mode	Wave number(cm-1)
A	H-O-H	3433.613
B	Si-O	1081.915
C	P-O	952.407
D	Si-O(NBO)	796.208

The IR spectra (Fig. 14and15) after 4 and 16 days in SBF were markedly changed in the case of BG and BG-.5 Ce, the bands assigned to Si O (2NBO) and Si–O (NBO) disappeared and those due to Si–O–Si group were still present, although very weak, indicating the polymerization of the Si–OH groups formed in the initial stages o fraction as a results of the ion exchange with H^+ of the solution. In addition we observed new bands in the spectral region typical of C–O and P–O vibrations in agreement with the presence of crystalline apatite revealed by XRD techniques. The spectra of BG-2, and BG-7 Ce showed lower modifications and still showed the bands assigned to Si–O (2NBO) and Si– O (NBO) and bands assignable to the P–O vibrations of an apatite crystalline phase were not detected. On the basis of the position of these bands we may propose that in these cases the original glass structure was maintained.

6. Conclusions

- The formation of cerium-containing phosphosilicate glasses is obtained until addition of 8% of CeO2. The addition of small quantities of CeO2 up to 0.5% to Bio-glass 45S5 does not alter significantly its ability of in vitro apatite formation within few days of immersion in SBF.High cerium content improves the chemical durability of glasses so the reactivity is negatively affected and cerium-containing phosphates seem to be preferred with respect to calcium containing ones. The improvement of glass durability is ascribed to the degree of covalent character of Ce–O bond and the hydrolytic dissociation is difficult. The apatite formation is prevented both by glass durability and by cerium ability to interact with phosphate giving rise to an amorphous phase. The cerium concentration is always extremely low and the ion is immobilized in a solid phase.
- Increasing the CeO_2 from 0-8 mole % causes an increase in density as well as compressive strength of the bio-glass
- No sharp peak was found. So the prepared bio-glass samples were non-crystalline in nature.
- Absorption bands of O-H and P-O reflecting the vibrations of OH and PO_4 groups in the bio-glass sample have been found at different wave numbers

7. References

[1] L.L. Hench, R.J. Splinter, W.C. Allen, T.K. Greenlee Jr.,J. Biomed. Mater. Res. 2 (1972) 117.
[2] L.L. Hench, J. Am. Ceram. Soc.74(1991) 1487, and references therein.
[3] U. Gross, R. Kinne, H.J. Schimit, V. Strunz, CRC Crit.Res. Biocompatible. 4(1988) 2.
[4] H.M. Kim, F. Miyaji, T. Kokubo, J. Am. Ceram. Soc. 78(1995) 2405, and references therein.
[5] T. Kokubo, M. Shigematsu, Y. Hagashima, T. Tashiro, T.Nakaura, T. Yamamuro, H. Higashi, Bull. Inst. Chem.
[6] G.H. Beall, K. Chyung, H.J. Watkins, Fluormica Glass-Ceramics, 1974, US Patent US-PS 3,801, 295 April 2.
[7] W. Vogel, W. Holland, K. Neumann, J. Gummel, J. Non- Cryst. Solids
[8] P. Stoor, E. S€oderling, J.I. Salonen, Acta Odontol. Scan
[9] N. Irving Sax, R.J. Lewis Sr., in: Dangerous Properties of Industrial Materials, 7th Ed., vol. 2, Van Nostrand
[10] Hench LL, Splinter, RJ, Allen WC, Greenlee TK. Bonding mechanisms at the interface of ceramic prosthetic materials. J. Biomed. Mater. Res. Symp.1971; 2 (Part I): 117–141
[11] Wilson J, Nolletti D. In Handbook of Bioactive Ceramics. Yamamuro T, Hench LL, Wilson J Editors. CRC Press, Boca Raton, FL. 1990: 283.
[12] Hench LL. In Bioceramics: Materials characteristics versus in-vivo behavior. Annals of New York Acad. Sci. New York 1998; 523: 54.